PHYSICAL EVIDENCE–FORENSIC SEROLOGY UNIT

QUALITY MANUAL

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1 SCOPE

This manual follows the requirements specified by ANSI-ASQ National Accreditation Board (ANAB), which is based on the ISO/IEC 17025:2017 standards and the 2017 ANAB ISO/IEC 17025:2017 — Forensic Science Testing and Calibration Laboratories Accreditation Requirements (AR 3125).

This *Physical Evidence-Forensic Serology Unit Quality Manual* is written specifically for the analysts working in the Physical Evidence Section that perform analysis in Body Fluid Identification.

Evidence selection for analysis is based on the analyst's training and experience.

This manual follows the outline of the *ASCL Quality Manual* (ASCL-DOC-01).

Other Supporting Manuals include:

- *ASCL Personnel Handbook* (ASCL-DOC-02) includes State, Federal, and ASCL policies
- *ASCL Health and Safety Manual* (ASCL-DOC-08) contains safety and environmental compliance policies and information
- *Serology Training Manual* (SER-DOC-02)

1.1 INTERNATIONAL STANDARD: GENERAL REQUIREMENTS

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding the International Standard: General Requirements.

1.2 INTERNATIONAL STANDARD: SCOPE

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding the International Standard: Scope.

1.2.1 ANAB PROGRAM

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding the ANAB program.

The Arkansas State Crime Laboratory is accredited through ANAB in the disciplines and categories of testing listed in its scope. The Serology Unit scope is listed in the *ASCL Quality Manual* (ASCL-DOC-01).

2 NORMATIVE REFERENCES

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding normative references.

3 TERMS AND DEFINITIONS

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding terms and definitions.

In addition to the terms and definitions listed in the *ASCL Quality Manual* (ASCL-DOC-01), terms commonly used in the Forensic Serology Unit are listed below.

SEXUAL ASSAULT KIT

A set of items used by medical personnel for gathering and preserving physical evidence following an allegation of sexual assault or rape.

SEMEN

The entire male ejaculate, typically including sperm cells and secretions from various glands in the male reproductive system.

SPERM CELLS (SPERMATOZOA)

Reproductive cells originating from the testes of a male individual.

Note: Spermatozoa will not be present in semen from males with azoospermia or from males that have undergone successful vasectomies.

P30 (AKA PROSTATE SPECIFIC ANTIGEN)

A protein produced by the male prostate gland which is expelled as one of the components of semen.

BLOOD

A specialized body fluid composed of plasma and cells (red blood cells containing hemoglobin, white blood cells, and platelets).

SUBSTRATE CONTROL

A sample collected from a non-stained area of an item of evidence.

PRESUMPTIVE (SCREENING) TEST

A preliminary screening test that is very sensitive, but not entirely specific to a certain body fluid.

Examples: Phenolphthalein Blood Indicator Test and BCIP (Acid Phosphatase) Screening Test

CONFIRMATORY TEST

A test that establishes the identity of a specific biological material or body fluid.

Examples: Takayama Blood Test, ABAcard® HemaTrace® test, Microscopic Identification of Spermatozoa, and the SERATEC® PSA Semiquant test

TAPE LIFTS

Adhesive tape used to lift hairs, fibers, cellular materials, and other foreign materials from the surface of evidence.

COMMONLY USED ABBREVIATIONS

- NOVN – nothing of value noted
- TL – tape lift
- SASAEC – State of Arkansas Sexual Assault Evidence Collection
- QNS – quantity not sufficient
- ANPT – all necessary precautions taken
- B – blood
- S – semen
- T – touch or transfer DNA
- ASNE – also submitted, not examined

Note: A Master Abbreviation List for the Serology Unit (SER-DOC-03) can be viewed on Qualtrax®.

4 GENERAL REQUIREMENTS

4.1 IMPARTIALITY

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding impartiality.

4.2 CONFIDENTIALITY

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding confidentiality.

Investigative information may not be released until after a technical review has been completed. However, one exception in the Physical Evidence-Forensic Serology Unit Quality Manual allows release of such information after an independent verification: The verification of sperm cells by a qualified analyst.

4.2.1 STATUTE

Case information at the ASCL is controlled by state statute (§ 12-12-312). This includes all case information obtained or created during the performance of laboratory activities.

Records, files, and information kept, obtained, or retained by the ASCL are privileged and confidential. However, the ASCL shall grant access to records pertaining to a defendant's criminal case to:

- the defendant,
- the public defender or other attorney of record for the defendant,
- the prosecuting attorney or deputy prosecuting attorney having jurisdiction over the criminal case, and
- to another party at the direction of
  - a court of competent jurisdiction, or
  - the prosecuting attorney having criminal jurisdiction over the case

Customer agencies that have made the necessary arrangements with the ASCL are granted secure access to JusticeTrax® iResults, where they may check on the status of their laboratory requests and view completed reports for their agency. JusticeTrax® access is secured by username/password.

Investigative information may not be released until after a technical review has been completed[1]. Final results, conclusions, or reports will be released only after a technical and administrative review of the case file has been completed and documented.

[1] Sperm cell identification/verification is the only exception to this in Serology.

4.2.2 THIRD-PARTY RELEASE

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding third-party release.

4.2.3 THIRD-PARTY SOURCE

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding third-party source.

4.2.4 SCOPE OF CONFIDENTIALITY

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding scope of confidentiality.

5 STRUCTURAL REQUIREMENTS

5.1 ESTABLISHMENT

Act 517 of 1977 established the Arkansas State Crime Laboratory (ASCL) via A. C. A. § 12-12-301.

5.2 MANAGEMENT

The Arkansas State Crime Laboratory is managed by the Director, who has overall responsibility for the laboratory.

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding labwide management.

5.2.1 OTHER STAFF (PHYSICAL EVIDENCE–FORENSIC SEROLOGY UNIT STAFF)

5.2.1.1 PHYSICAL EVIDENCE SECTION CHIEF

QUALIFICATIONS

The position requires a minimum of a baccalaureate or an advanced degree in a chemical, physical, or biological science, or forensic science plus five years of experience in a forensic laboratory. Other job-related education and/or experience may be substituted for all or part of these basic requirements upon approval of the Assistant Director. The Physical Evidence Section Chief or designee (e.g., a Quality Manager or a Technical Leader) will have the appropriate technical training and experience in all disciplines encompassed by the section.

AUTHORITIES AND RESPONSIBILITIES

- The Physical Evidence Section Chief is under administrative direction and is responsible for directing the activities of the Physical Evidence Section. The Physical Evidence Section Chief has overall responsibility for the technical operations and the provisions of the resources needed to ensure the required quality of laboratory operations.
- Supervises a professional staff of Serologists and Trace Evidence Analysts by interviewing and recommending for hire; training or providing training opportunities; assigning and reviewing work; and evaluating the performance of incumbents.
- Coordinates section activities by reviewing, prioritizing, and assigning new cases; providing assistance to staff in regard to appropriate testing methods and findings; and reviewing selected final reports.

- Reviews investigator's summary sheet to become familiar with the details of the crime, reviews items submitted to determine appropriate testing methods, and assigns cases to appropriate personnel.
- Conducts a series of analytical tests, prepares reports of findings and conclusions, and testifies in court as an expert witness.
- Writes articles, presents training, and provides consultation to law enforcement officers, prosecutors, defense attorneys, and other public officials on crime scene investigation and methods of collecting, transporting and preserving, evidence to ensure its integrity and maintenance of the chain of custody.
- Researches scientific literature and exchanges information with peers in other states in order to stay abreast of the latest scientific advances in the analysis of criminal evidence and/or determine the best method of testing a particular piece of evidence.
- Performs administrative duties by preparing activity reports, inventory reports; maintaining employee history information and equipment maintenance logs; requisitioning supplies and equipment; and researching and recommending policies/procedures.
- Assists law enforcement agencies with on-site crime scene investigations after gaining approval from the Director or the Assistant Director. Any assistance provided is under the direction of the law enforcement agency in charge of the crime scene.
- Performs related responsibilities as required or assigned.
- May delegate duties as required.
- Ensures compliance with the requirements specified by ANSI-ASQ National Accreditation Board (ANAB), which is based on the ISO/IEC 17025:2017 standards and the 2017 ANAB ISO/IEC 17025:2017 — Forensic Science Testing and Calibration Laboratories Accreditation Requirements (AR 3125).
- Appoints deputies for key management personnel when the section chief will be absent for 3 days or longer. All affected personnel shall be notified.
- Ensures that employees are notified of their responsibilities and expectations concerning the objective of the ASCL quality system and provide feedback on actual job performance.
- Conveys information concerning the quality system to Physical Evidence analysts.

WORKING RELATIONSHIPS

- The Physical Evidence Section Chief has regular contact with other laboratory sections, law enforcement officials, attorneys, criminal/civil court personnel, and peers in other states.

SPECIAL JOB DIMENSIONS

- The Physical Evidence Section Chief will experience frequent exposure to hazardous, toxic, repulsive, and/or infectious materials. Occasional in or out-of-state travel and on-call duty are required.

5.2.1.2 FORENSIC SEROLOGIST

QUALIFICATIONS

The position requires a minimum of a baccalaureate or an advanced degree in a chemical, physical, or biological science, or forensic science.

AUTHORITIES AND RESPONSIBILITIES

- Reviews investigator's summary information to become familiar with the details of the crime and examines items of evidence to determine appropriate testing methods.
- Conducts a series of analytical tests to identify biological fluids and locate areas that may be suitable for DNA testing. Hairs and fibers (tape lifts) may be collected for future analyses.
- Prepares reports of findings and conclusions for submission to legal authorities and courts of law.
- Testifies in court as an expert witness on the analysis of evidence and conclusions reached.
- Writes articles, presents training, and provides consultation to law enforcement officers, prosecutors, defense attorneys, and other public officials on crime scene investigation concerning methods of collecting, transporting, and preserving evidence to ensure its integrity and maintenance of the chain of custody.
- Researches scientific literature and exchanges information with peers in other states in order to stay abreast of the latest scientific advances in the analysis of criminal evidence and/or determine the best method of testing a particular piece of evidence.
- Assists law enforcement agencies with on-site crime scene investigations after gaining approval from the Physical Evidence Section Chief. Any assistance provided is under the direction of the law enforcement agency in charge of the crime scene.
- Performs related responsibilities as required or assigned.

WORKING RELATIONSHIPS

- The Forensic Serologist has regular contact with other laboratory sections, law enforcement officials, attorneys, and peers in other states.

SPECIAL JOB DIMENSIONS

- Forensic Serologists will experience frequent exposure to hazardous, toxic, repulsive, and/or infectious materials. Occasional in or out-of-state travel and on-call duty are required.

5.2.1.3 FORENSIC SEROLOGY UNIT QUALITY MANAGER

The Forensic Serology Unit will have a Quality Manager. The individual will be responsible for ensuring that the management system related to quality is implemented and followed at all times.

Other responsibilities include, but are not limited to:

- Maintaining and updating Serology Unit manuals and documents.
- Monitoring section practices to verify continuing compliance with policies and procedures.

- Maintaining and evaluating unit maintenance records and periodically assessing the adequacy of report review activities.
- Ensuring the proper validation of new technical procedures.
- Performs QC on purchased reagents and supplies, when applicable
- Conducts performance checks when needed
- Maintains key log

5.2.1.4 FORENSIC SEROLOGY UNIT TRAINING OFFICER

The Forensic Serology Unit will have at least one Training Officer. The individual(s) will be responsible for ensuring that all newly-hired analysts (as well as any analysts undergoing cross-training in Serology) receive the proper training required for independently functioning as a Forensic Serologist.

Other responsibilities include, but are not limited to:

- Preparing practice cases for serological testing.
- Monitoring the trainee's progress and adjusting the length of the training program if deficiencies are observed. Should concerns arise, they should be communicated to the Physical Evidence Section Chief.
- Providing feedback to the trainee throughout the training program.
- Preparing competency exam items for serological testing.
- Administering and grading quizzes.

5.2.1.5 FORENSIC SEROLOGY UNIT HEALTH AND SAFETY MANAGER

The Forensic Serology Unit will have a Health and Safety Manager. The individual will be responsible for ensuring that the management system related to health and safety is implemented and followed at all times.

Other responsibilities include, but are not limited to:

- Conducting monthly safety inspections and ensuring that proper practices and procedures are being followed within the designated area.
- Maintaining records of any safety incidents within the designated area.
- Maintaining a current copy of the SDSs used within the designated area.
- Working with the labwide Health and Safety Manager to seek ways to improve the safety program.

5.3 SCOPE OF LABORATORY ACTIVITIES–SEROLOGY UNIT

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding scope of laboratory activities for the Serology Unit.

This *Physical Evidence – Forensic Serology Unit Quality Manual* is written specifically for the analysts working in the Forensic Serology Unit and performing analyses in the following areas:

- Body Fluid Identification
- Collection of Hairs and Fibers
- Collection of Stains for Further Testing

Every case is unique and must be evaluated by the individual examiner. Neither all of the possible analyses encountered nor decisions to be made in casework can be appropriately covered in a procedure manual nor can all of the possible variations be described. This quality manual is written to serve as a guideline for a majority of casework encountered in the Serology Unit and to help the analyst choose the best analytical scheme for the evidence submitted.

5.4 NORMATIVE DOCUMENTS

This manual follows the requirements specified by the ANAB Accreditation Program, which includes conformance to:

- Arkansas Code Annotated (A.C.A.) 12-12-301 through 12-12-326
- ISO/IEC 17025:2017 (*General requirements for the competence of testing and calibration laboratories*)
- ANAB AR 3125 (*ISO/IEC 17025:2017 Forensic Testing and Calibration Laboratories Accreditation Requirements*)
- Labwide and Physical Evidence-Forensic Serology Unit Quality Manuals
- Labwide and Physical Evidence-Forensic Serology Unit Section Training Manuals

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding normative documents.

5.5 LABORATORY OPERATIONS

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding laboratory operations.

5.6 QUALITY MANAGEMENT

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding quality management.

5.7 MANAGEMENT SYSTEM COMMUNICATION AND INTEGRITY

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding management system communication and integrity.

6 RESOURCE REQUIREMENTS

6.1 GENERAL

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding resource requirements.

6.2 PERSONNEL

6.2.1 GENERAL

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding personnel.

6.2.2 COMPETENCE REQUIREMENTS

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding competence requirements.

6.2.2.1 FORENSIC SEROLOGIST EDUCATIONAL REQUIREMENTS

Analysts who authorize results, opinions and/or interpretations in the Forensic Serology Unit shall meet these minimum educational requirements:

Discipline	Minimum Educational Requirement
Biology	A baccalaureate or an advanced degree in a chemical, physical, or biological science, or forensic science

6.2.2.2 TRAINING PROGRAM

The Forensic Serology Unit training program is detailed in the *Serology Training Manual* (SER-DOC-02). The training program covers all aspects of training, to the extent necessary, based on the job function. Analysts-in-training will only be allowed to handle case evidence and conduct serological testing while under supervision by a qualified serologist after their training manual has been signed off on in key areas of the training modules which covers those tasks.

The Forensic Serology Unit training program requires analysts-in-training to work with qualified analysts on a daily basis for a minimum of six months[2]. Additional time may be required of specific employees.

Note: It is understood that interruptions (e.g., court testimony, illness) may limit the analysts' daily

[2] Occasionally, less time may be required based on an analyst's previous training or if partial serology training is planned. These exceptions will be authorized by the Physical Evidence Section Chief and documented in the Serology Training Manual.

availability to the trainee. The training officer should be mindful of the total quantity of interrupted days in regard to the six month minimum time frame required for training and extend the end date of training accordingly if substitute trainers could not be arranged on those affected days.

Serologists-in-training will begin by observing casework with a qualified analyst. The serologist will be instructed in the following areas during his or her training:

- Evidence assessment
- Hair and fiber collection
- Alternate light source (ALS) use
- Stain collection
- Collection of samples for transfer
- Blood examination
- Semen examination
- Report writing

The serologist-in-training will be instructed in the following components of evidence assessment:

- Assessment of the information provided by the customer
- Assessment of the evidence submitted for examination in light of the information provided
- Determining whether the evidence submitted may have probative value for testing by other sections
- Ensuring the integrity of evidence for testing by other sections such as, but not limited to, Latent Prints, Trace Evidence, and Firearms and Tool Marks

DNA ANALYST SHADOWING

When a DNA analyst shadows Serologists as part of the DNA training program, they shall be administered a competency examination by the Serology Training Officer **before** being allowed to handle case evidence or conduct testing on case evidence under the supervision of a qualified serologist. Copies of this examination documentation will be kept in the DNA analyst's training binder as well as on the S: drive in Serology.

ADDITIONAL TRAINING

The training program shall include the application of ethical practices in forensic science, a general knowledge of forensic science, and applicable criminal and civil law procedures.

Serologists-in-training will be evaluated through observation, practice testing, and verbal questioning by the training officer(s), other analysts, and the Physical Evidence Section Chief.

The *Serology Training Manual* (SER-DOC-02) states objectives, specific reading requirements, tasks, and practical exercises for analysts to complete during the training period.

Upon completion of the training program, the serologist-in-training will demonstrate his or her competency in Serology by completing the following tasks:

1) Take a written exam to demonstrate his or her knowledge of proper evidence handling, serological testing, and the analytical procedures used in serological testing.
2) Perform an examination of unknown samples[3], which are representative of evidence encountered in casework, to demonstrate his or her proficiency in the following areas (where applicable):

- Evidence assessment
- Hair and fiber collection
- ALS use
- Stain collection
- Collection of samples for transfer
- Blood examination
- Semen examination
- Report writing
- Participate in moot court proceedings

MOOT COURT

Serologists-in-training participate in, at minimum, one moot court proceeding. Additional moot court proceedings are conducted when deemed necessary by the Physical Evidence Section Chief.

COMPETENCY RECORD

At the conclusion of training, the Section Chief shall document by memorandum to the Director and Quality Assurance Manager that the individual has been properly trained and that their ability to perform the specified testing has been assessed. This record shall be kept in the individual's Training Binder and in Qualtrax® in the Training section of the Personnel tab. In addition, the Analyst and Technician Competency Authorization Documentation form (ASCL-FORM-62) must be completed (or updated) and recorded in the Personnel tab of Qualtrax®.

NEW PROCEDURE TRAINING

Should new procedures be implemented, training shall be documented for existing employees. Training shall include observation of the method and, if appropriate, a proficiency test administered. Both observation and completion of a proficiency test shall be documented in the employee's training binder.

Records of training will be kept in the employee's training binder. Records of authorizations will be maintained in the Personnel tab in Qualtrax®.

[3] Known as a competency test

6.2.3 COMPETENCE OF STAFF

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding the competence of staff.

The Physical Evidence Section Chief ensures the competence of all personnel to perform the tasks for which they are responsible, and to evaluate the significance of any deviations from policy and/or procedure.

6.2.3.1 COMPETENCY TESTING

All serologists who perform testing[4] are competency tested. This competency test includes practical examination(s) of an unknown sample (or samples) that cover the spectrum of anticipated testing tasks. Competency testing may be conducted on a single task or a group of tasks, as appropriate.

For laboratory personnel whose job responsibility includes report writing, a competency test shall include, at a minimum:

- Practical examination of sufficient unknown samples to cover the anticipated spectrum of assigned testing tasks, to evaluate the individual's ability to properly perform analysis
- A written report to demonstrate the individual's ability to properly convey results and/or conclusions and the significance of those results and/or conclusions
- A written or oral examination to assess the individual's knowledge of the discipline, category of testing, or task being performed, and
- Moot court[5] to demonstrate the individual's ability to properly convey and present results of evidence in court

The intended result(s) of the competency test shall be achieved and documented prior to performing the covered task(s) on actual items of evidence. This may be achieved in several ways, including:

- Observed testing on a surrogate item[6]
- Written examination
- Oral examination

The risk involved will be considered when determining the extent of the competency test.

6.2.3.2 COMPETENCY-TESTED ACTIVITIES

Competency testing for the following activities will be conducted and documented prior to these actions being performed on evidence:

- Laboratory activities (testing and/or sampling)

[4] i.e., laboratory activities (analysis/examination of evidence) and/or analysis of results
[5] The requirement for moot court may be waived for employees receiving training in additional categories of testing within the same discipline
[6] Such as a secondary standard or old proficiency test material

- Analysis of results
- Review of results
- Authorization of results
- Verification of results
- Technical review
- Expressing an opinion or interpretation

EMPLOYEE DEVELOPMENT PROGRAM

Serologists participate in annual continuing education. This may include professional meetings, staff development seminars, technical training courses, in-house technical meetings, courses and seminars, or ASCL sponsored seminars and conferences. Continuing education may also be achieved through online course/training offerings. Continuing education participation shall be documented in Qualtrax®.

6.2.4 DUTIES, RESPONSIBILITIES, AND AUTHORITIES

The duties, responsibilities, and authorities of each position in the Forensic Serology Unit are contained in section 5.2.1 of this manual.

6.2.5 PERSONNEL REQUIREMENTS

See *ASCL Quality Manual* (ASCL-DOC-01) for procedures and record retention information.

JOB DESCRIPTIONS

Current job descriptions for court-qualified analysts shall be maintained in Qualtrax®.

LITERATURE

Analysts and trainees are encouraged to read current literature regularly; a Literature Review Log is maintained in Qualtrax® for Physical Evidence Section analysts and trainees to document their survey of forensic literature.

6.2.6 AUTHORIZATIONS

The Physical Evidence Section Chief will authorize personnel to perform certain duties. Personnel may not perform these duties without authorization, except during supervised training, when applicable. These duties include:

- Performing testing activities
  - Use of equipment (as applicable)
- Method development, modification, verification, and/or validation
- Analysis of results, including:
  - Statements of conformity
  - Opinions/interpretations

- Reporting results
- Reviewing Results
- Authorizing results

Authorization documentation shall be part of the competency documentation and shall be dated and signed by the Physical Evidence Section Chief upon completion of training and is maintained in Qualtrax®. See (ASCL-FORM-62) *Analyst & Technician Competency Authorization Documentation.* Each Serologist's Qualtrax® record shall also contain a curriculum vitae or résumé that includes educational and professional qualifications, training, skills, and experience. The individual's Training Binder will contain all completed training records.

6.3 FACILITIES AND ENVIRONMENTAL CONDITIONS

6.3.1 GENERAL

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding facilities and environmental conditions.

The Physical Evidence-Forensic Serology Unit performs laboratory activities at the Main Laboratory which is located at 3 Natural Resources Drive, Little Rock AR 72205.

6.3.2 DOCUMENTATION

ENVIRONMENTAL CONDITIONS

Temperature can affect the quality of results when using certain reagents and critical supplies for particular procedures in the Forensic Serology Unit. As a safeguard against unexpected temperature fluctuations in the (normally climate controlled) Physical Evidence lab area(s) outside of business hours, these reagents and supplies must be refrigerated when not in use. When reagents and supplies need to be refrigerated, the temperature of the refrigeration unit will be monitored, controlled, and recorded. Refer to Table 6 in section 6.4.4 of this manual.

6.3.3 MONITORING RECORDS

The Forensic Serology Unit will monitor and record environmental conditions if:

- These conditions are specified in a method or procedure, or
- The conditions influence the validity of results
- See SER-FORM-30 *Instrument Maintenance and Temperature Log* for each refrigerator maintained by the Forensic Serology Unit for reagent, supply, and/or biological sample storage

and for each water bath maintained by the Forensic Serology Unit for acid phosphatase testing (presumptive screening of suspected seminal stains). These forms are located in the Instrument Maintenance and Temperature Log Binders for each lab area where serological testing is conducted.

- Refer to the individual reagent forms listed under the Physical Evidence-Serology Discipline folder on Qualtrax® for any refrigeration requirements.

6.3.4 CONTROL OF FACILITIES

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding the control of facilities.

6.3.4.1 ACCESS

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding access.

A locked key box is located within the Physical Evidence Section and the Physical Evidence Section Chief and the Quality Manager(s) have access to the key box. A key log is maintained by the Quality Manager(s) on the S: drive.

6.3.4.2 PREVENTION OF ADVERSE INFLUENCES

Forensic Serologists are responsible for taking the necessary measures to prevent contamination, cross-contamination, interference, or adverse influences on laboratory activities.

CONTAMINATION PREVENTION PROCEDURES

The Serology Unit uses appropriate methods for all testing and evidence handling that meet the needs of the customer which includes specific contamination prevention procedures. Refer to section 9.1 for details.

6.3.4.3 SEPARATION

The Forensic Serology Unit's laboratory areas are designed to ensure effective separation between neighboring areas in which there are incompatible activities (e.g., examination of evidence from different scenes, examination of evidence from different individuals). The Unit contains multiple work areas for the examination of evidence (e.g., separate scrape-down rooms) which shall be used for examination of such items as deemed necessary. Examinations shall be separated by location and/or time when items are not from the same individual or are not from the same scene location.

6.3.5 EXTERNAL ACTIVITIES

When the Forensic Serology Unit performs laboratory activities at sites or facilities outside its permanent control, it shall ensure that the requirements related to facilities and environmental conditions of this document are met.

6.4 EQUIPMENT

6.4.1 ACCESS

The Forensic Serology Unit has adequate equipment to perform all necessary testing. Details of specific quality control measures on equipment that have a significant effect on the quality of test results, if applicable, will be outlined in this manual.

6.4.2 OUTSIDE EQUIPMENT

If the Forensic Serology Unit must use equipment outside of its permanent control, it shall ensure that the equipment meets the requirements of this section.

A successful performance verification is required for any equipment that has gone outside of the direct control of the laboratory (e.g., for repair or preventive maintenance) before that equipment may be returned to service. Documentation of these verifications will be maintained.

6.4.3 PROPER FUNCTIONING

The Forensic Serology Unit has procedures for equipment, when applicable, to ensure proper functioning and prevent contamination or deterioration, including:

- Handling
- Transport
- Storage
- Use
- Planned maintenance

See section 9 Serology Unit Test Methods for specific procedural information.

The Serology Unit has adequate equipment to perform the necessary testing. The equipment is maintained by the personnel in the discipline who use it.

SEROLOGY EQUIPMENT

- Water Bath
- Alternate Light Source
- Centrifuge
- Oven
- View Box
- Refrigerator
- Microscope
- Analytical Balance

- pH Meter
- Ductless Hood
- Reference Materials (e.g., Blood and Semen Standards)
- ABAcard® HemaTrace® (**Critical Supply**)
- SERATEC® PSA-Semiquant (**Critical Supply**)
- Chemicals and Reagents (See Qualtrax® and/or Serology Reagent Prep Logbook for names/formulations.)
- Disposable pipets & glassware[7]

New employees shall be trained on the appropriate equipment during their training program, as detailed in the Forensic Serology Unit Training Manual. The Physical Evidence Section Chief shall authorize personnel to operate equipment (documented on *Analyst & Technician Competency Authorization Documentation,* ASCL-FORM-62). This authorization documentation is maintained in Qualtrax®. Only individuals who have been trained in the proper use of the equipment shall operate it.

NEW EQUIPMENT

When new equipment requires a validation, appropriate personnel will be trained in its use. This training will be documented and maintained in Qualtrax®.

Up-to-date instructions on the use and maintenance of the equipment shall be readily available for use.

REAGENTS/CHEMICALS/CONTROLS (STANDARDS)

Reagents, chemicals, and controls (standards)[8] used by the Serology Unit are maintained and quality controlled.

In addition, the following rules shall be followed:

- Items with a manufacturer-specified expiration date may not be used after that date without documentation to support continued reliability.
- For items without a manufacturer-specified expiration date, dates will be based on experience, industry standard, or scientific consensus.
- Appropriate logs must be maintained within each discipline for reagents and standards (reference materials) used.
- Each analyst must ensure that the controls (standards), reagents, and chemicals used in their analysis are of satisfactory quality.
- Controls (standards), reagents, or chemicals that are determined not to be reliable must be immediately removed from use.

[7] Examples: graduated beakers and cylinders used to prepare serological reagents.
[8] Examples of standards: Blood standard and semen standard used for daily QC checks. They may be referred to as "positive controls" by serologists due to footer labeling on serological worksheets.

- Directions for the preparation of commonly used reagents are found in the Reagent Logbook and on Qualtrax®.

6.4.3.1 REAGENT RECORDS AND LABELING

DOCUMENTATION AND LABELING

Reagents may be purchased or prepared. Minimum requirements for quality control of reagents are outlined below.

PURCHASED REAGENTS/CHEMICALS

Containers must be labeled with the following:
- Lot number
- Date opened
- Expiration date (if applicable)
- Initials upon opening
- Date received and initials

PREPARED REAGENTS

Containers must be labeled with the following:
- Identity of reagent
- Date of preparation
- Date of expiration
- Initials of preparer
- Lot number—Takayama ONLY

Note: Aliquot containers/bottles of prepared reagents (e.g., Takayama aliquot bottles) should be labeled with the same information as the original container/bottle, including the initials of the original preparer.

PREPARED REAGENTS

Logbook must include the following:
- Identity of reagent
- Date of preparation
- Date of expiration
- Instructions on preparation of reagent
- Lot numbers of solvents and/or chemicals used in preparation of reagent
- A method to verify the reagent's reliability *
- Initials of the person preparing reagent
- Initials of the person verifying reagent (if applicable)
- Date reagent in use

*The reliability testing shall occur before use or, if appropriate, concurrent with the test.

CONTROLS

- Specifications of appropriate controls are discussed in the Test Methods portion of this manual (See section 9).

6.4.3.2 REFERENCE COLLECTION RECORDS

There are no reference collections maintained by the Forensic Serology Unit.

6.4.4 PERFORMANCE VERIFICATION

Before equipment is placed into service or returned into service, a performance verification will be successfully completed. This ensures that the equipment meets all specified requirements.

Designated equipment will also be subject to a schedule of performance verifications. All performance verifications shall be properly documented in the (SER-FORM-30) *Instrument Maintenance and Temperature Log*. This log shall be maintained and readily available to each analyst who utilizes it. See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding performance verification.

WATER BATH PERFORMANCE VERIFICATION & MAINTENANCE (PREVENTATIVE)

An *Instrument Maintenance and Temperature Log* is provided for each water bath in use in the Serology laboratory. Each water bath is subjected to the temperature performance checks (see TABLE 1) on a monthly basis. The results of the checks will be recorded on the appropriate log sheet. Log sheets are filed and archived generally on a yearly basis or when binder becomes full.

The analyst will check the temperature of the water bath at each use to verify that the temperature is within the specified temperature range of 37˚C ±5˚C. This pass/fail verification will be recorded on the examination worksheet.

Should an analyst encounter a problem with the water bath during use, the "Troubleshooting Checks" provided in TABLE 1 will assist the analyst in determining the problem so it may be corrected. Any maintenance resulting from a Troubleshooting Check will be recorded on the appropriate log sheet.

TABLE 1 Routine Monthly Water Bath Checks and Troubleshooting Guide	
Monthly Checks	**Actions**
Check water bath temperature	Record temperature
Is the water temperature 37˚C ±5 ˚C?	If no, adjust temperature setting on water bath, document in maintenance log[9] If yes, the water bath is ready to use
Troubleshooting Checks	**Actions**
Is water level sufficient for testing?	Add water as necessary (It is not necessary to document when water is added to the water bath)

See TABLE 9 in section 6.4.13 for the water bath maintenance requirements.

ALTERNATE LIGHT SOURCE PERFORMANCE VERIFICATION & MAINTENANCE (PREVENTATIVE)

An *Instrument Maintenance and Temperature Log* is provided for each alternate light source in use in the Serology sections. The alternate light source does not require regular performance verification. Log sheets are filed and archived generally on a yearly basis or when binder becomes full.

Should an analyst encounter a problem with the alternate light source during use, the "Troubleshooting Checks" provided in TABLE 2 will assist the analyst in determining the problem so it may be corrected. Any maintenance resulting from a Troubleshooting Check will be recorded on the appropriate log sheet.

See TABLE 9 in section 6.4.13 for the alternate light source maintenance requirements.

TABLE 2 ALTERNATE LIGHT SOURCE Troubleshooting Guide	
Troubleshooting Checks	**Actions**
Is light bulb damaged?	If damaged, replace bulb, document in maintenance log
Is the wavelength set to 450nm?	Adjust as necessary
Are the correct lenses being used?	Yellow lenses are recommended at 450nm (Amber/orange lenses, if using Rofin)

If any of the above actions fail to correct the problem then the alternate light source must be removed from service for repair/replacement. After the alternate light source is repaired/replaced, the alternate light source should be checked to ensure proper functionality and wavelength. All

[9] If the water bath temperature remains outside acceptable temperature range after adjustment, the water bath must be removed from service for repair/replacement. After water bath is repaired/replaced, water bath temperature must be checked prior to return to service. All repairs and maintenance must be documented on the *Instrument Maintenance and Temperature Log*.

repairs and maintenance must be documented on the *Instrument Maintenance and Temperature Log*.

CENTRIFUGE PERFORMANCE VERIFICATION & MAINTENANCE (PREVENTATIVE)

An *Instrument Maintenance and Temperature Log* is provided for each centrifuge in use in the Serology sections. The centrifuge does not require regular performance verification. Log sheets are filed and archived generally on a yearly basis or when binder becomes full.

Should an analyst encounter a problem with the centrifuge during use, the "Troubleshooting Checks" provided in TABLE 3 will assist the analyst in determining the problem so it may be corrected. Any maintenance resulting from a Troubleshooting Check will be recorded on the appropriate log sheet.

See TABLE 9 in section 6.4.13 for the centrifuge maintenance requirements.

TABLE 3 CENTRIFUGE Troubleshooting Guide	
Troubleshooting Checks	**Actions**
Is the centrifuge on a level surface?	If no, place on a level surface
Does the centrifuge shake during use?	If yes, balance tubes
Does a pellet form after use?	If no, check speed and time settings

If any of the above actions fail to correct the problem then the centrifuge must be removed from service for repair/replacement. After the centrifuge is repaired/replaced, the centrifuge should be checked to ensure proper functionality. All repairs and maintenance must be documented on the *Instrument Maintenance and Temperature Log*.

OVEN PERFORMANCE VERIFICATION & MAINTENANCE (PREVENTATIVE)

An *Instrument Maintenance and Temperature Log* is provided for each oven in use in the Serology sections. The oven does not require regular performance verification. Log sheets are filed and archived generally on a yearly basis or when binder becomes full.

Should an analyst encounter a problem with the oven during use, the "Troubleshooting Checks" provided in TABLE 4 will assist the analyst in determining the problem so it may be corrected. Any maintenance resulting from a Troubleshooting Check will be recorded on the appropriate log sheet.

See TABLE 9 in section 6.4.13 for the oven maintenance requirements.

TABLE 4 OVEN Troubleshooting Guide	
Troubleshooting Checks	**Actions**
Is it warm?	If no, adjust temperature If yes, oven is fit for use

If any of the above actions fail to correct the problem then the oven must be removed from service for repair/replacement. After the oven is repaired/replaced, the oven should be checked to ensure proper functionality. All repairs and maintenance must be documented on the *Instrument Maintenance and Temperature Log*.

VIEW BOX PERFORMANCE VERIFICATION & MAINTENANCE (PREVENTATIVE)

An *Instrument Maintenance and Temperature Log* is provided for each view box in use in the Serology sections. The oven does not require regular performance verification. Log sheets are filed and archived generally on a yearly basis or when binder becomes full.

Should an analyst encounter a problem with the view box during use, the "Troubleshooting Checks" provided in TABLE 5 will assist the analyst in determining the problem so it may be corrected. Any maintenance resulting from a Troubleshooting Check will be recorded on the appropriate log sheet.

See TABLE 9 in section 6.4.13 for the view box maintenance requirements.

TABLE 5 VIEW BOX Troubleshooting Guide	
Troubleshooting Checks	**Actions**
Is it warm?	If no, adjust temperature If yes, view box is fit for use

If any of the above actions fail to correct the problem then the view box must be removed from service for repair/replacement. After the view box is repaired/replaced, the view box should be checked to ensure proper functionality. All repairs and maintenance must be documented on the *Instrument Maintenance and Temperature Log*.

REFRIGERATOR PERFORMANCE VERIFICATION & MAINTENANCE (PREVENTATIVE)

An *Instrument Maintenance and Temperature Log* is provided for each refrigerator in use in the Serology sections. Each refrigerator is subjected to the performance checks in TABLE 6 on a monthly basis. The results of the checks will be recorded on the appropriate log sheet. Log sheets are filed and archived on a yearly basis.

See TABLE 9 in section 6.4.13 for the refrigerator maintenance requirements.

TABLE 6 Routine Monthly Refrigerator Checks	
Monthly Checks	**Actions**
Check refrigerator temperature	Record temperature
Is the refrigerator temperature between 0 and 10°C?	If no, adjust temperature setting on refrigerator, document in maintenance log[10] If yes, the refrigerator is fit for use
Check freezer temperature (if applicable)	Record temperature
Is the freezer temperature between -9 and -21°C (if applicable)?	If no, adjust temperature setting on freezer, document in maintenance log[10] If yes, the freezer is fit for use

MICROSCOPE PERFORMANCE VERIFICATION & MAINTENANCE (PREVENTATIVE)

An *Instrument Maintenance and Temperature Log* is provided for each microscope in use in the Serology sections. The microscopes do not require regular performance verification. Log sheets are filed and archived generally on a yearly basis or when binder becomes full.

Should an analyst encounter a problem with the microscope during use, the "Troubleshooting Checks" provided in TABLE 7 will assist the analyst in determining the problem so it may be corrected. Any maintenance resulting from a Troubleshooting Check will be recorded on the appropriate log sheet.

See TABLE 9 in section 6.4.13 for the microscope maintenance requirements.

TABLE 7 MICROSCOPE Troubleshooting Guide	
Troubleshooting Checks	**Actions**
Is the light bulb damaged?	If damaged, replace bulb, document in maintenance log
Are the settings appropriate for analyst use?	Adjust as necessary, perform Köhler Illumination if needed

If any of the above actions fail to correct the problem then the microscope must be removed from service for repair/replacement. After the microscope is repaired/replaced, the microscope should be checked to ensure proper functionality. All repairs and maintenance must be documented on the *Instrument Maintenance and Temperature Log*.

[10] If the refrigerator or freezer temperature remains outside acceptable temperature range after adjustment, the unit must be removed from service for repair/replacement. After refrigerator is repaired/replaced, refrigerator and freezer (if applicable) temperature must be checked prior to return to service. All repairs and maintenance must be documented on the *Instrument Maintenance and Temperature Log*.

ANALYTICAL BALANCE PERFORMANCE VERIFICATION & MAINTENANCE

The DNA section maintains the performance verification of the analytical balances and performs any necessary maintenance. Any inefficiency in their performance will place the analytical balance in an out-of-service status until it can be serviced. Should a problem arise while using one of the analytical balances, the serologist will contact a member of the Forensic DNA section for assistance in troubleshooting.

PH METER PERFORMANCE VERIFICATION & MAINTENANCE

An *Instrument Maintenance and Temperature Log* is provided for the pH meter in use in the Serology section. The pH meter requires regular performance verification by calibrating[11] the instrument each time it is used. These calibrations are logged on the *Serology pH Meter Calibration Log* (SER-FORM-33). Log sheets are filed and archived generally on a yearly basis or when binder becomes full.

Should an analyst encounter a problem with the pH meter during use, the "Troubleshooting Checks" provided in TABLE 8 will assist the analyst in determining the problem so it may be corrected. Any maintenance resulting from a Troubleshooting Check will be recorded on the appropriate log sheet.

See TABLE 9 in section 6.4.13 for the pH meter maintenance requirements.

TABLE 8 pH Meter Troubleshooting Guide	
Troubleshooting Checks	**Actions**
Is the display blank?	Check batteries, replace if necessary, document in maintenance log
Is there an error on the display while attempting to calibrate?	Refer to pg. 16 Troubleshooting chart in pH meter Instruction Manual
Is there an error on the display while attempting to calibrate?	Check electrode buffer to verify adequate volume is present.

DUCTLESS HOOD PERFORMANCE VERIFICATION & MAINTENANCE (PREVENTATIVE)

An *Instrument Maintenance and Temperature Log* is provided for each ductless hood in use in the Serology laboratory. Maintenance and filter changes are performed on an as-needed basis. When maintenance or filter changes occur, they will be recorded on the appropriate log sheet. Log sheets are filed and archived generally on a yearly basis or when binder becomes full.

Should an analyst encounter a problem with the ductless hood during use (e.g. blower not turning on or audible alarm sounds), the analyst should reference Chapter 8 of the User's Manual for the ductless hood. A paper or electronic copy (thumb drive) is stored adjacent to each hood. Airflow

[11] Although the pH meter instruction manual refers to this process as "calibration," it is understood that this process is considered to be a performance verification/check to ensure the meter is functioning properly.

calibration will be conducted by an external service provider as needed. Any maintenance will be recorded on the appropriate log sheet.

REFERENCE MATERIALS

The Serology Unit has a procedure for the verification of its reference materials[12]. (See section 9.6.2 for the Semen Standard and section 9.10.2 for the Blood Standard.)

6.4.5 FITNESS FOR SERVICE

All equipment used for measurement will be capable of achieving the measurement accuracy and/or measurement uncertainty required to provide a valid result.

6.4.6 CALIBRATION REQUIREMENT

The Forensic Serology Unit does not use equipment that requires calibration.

6.4.7 CALIBRATION PROGRAM

The Forensic Serology Unit does not use equipment that requires calibration.

6.4.8 LABELLING

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding labelling of calibrated equipment or equipment that has a defined period of validity.

6.4.9 OUT OF SERVICE

Any equipment which has been subjected to overloading or mishandling, gives questionable results, or has been shown to be defective or outside specified requirements, shall be taken out of service.

It will be labeled as "Out of Service" or isolated from functional equipment to prevent its use. It will only be returned to service after it has been verified and documented to perform correctly. Surplus stored equipment will also be labeled as "Out of Service" and follow the same requirements as discussed above.

When equipment is removed from service because of a malfunction that has caused nonconforming work, a *Quality Assurance Concern* workflow is initiated in Qualtrax®, and the ASCL will examine any effect that the deviation may have had on its activities.

See section 7.10 for lab policies regarding nonconforming work.

[12] Blood and Semen Standards

6.4.10 INTERMEDIATE CHECKS

Intermediate checks of equipment may be necessary to maintain confidence in the performance of equipment. When necessary, these checks are conducted according to procedures documented in discipline quality manuals. These procedures are based upon a risk assessment, including such factors as:

- the calibration interval,
- the purpose of the equipment,
- the stability of the equipment,
- the requirements of the method(s), and
- the risk associated with a failed check.

6.4.11 CORRECTION FACTORS

The Forensic Serology Unit does not use calibration and reference material data that may include reference values or correction factors.

6.4.12 EQUIPMENT ADJUSTMENT

If unintended adjustments of equipment may influence testing results, the discipline will take precautions (when practicable) to prevent these unintended adjustments.

6.4.13 EQUIPMENT RECORDS

The *Instrument Maintenance and Temperature Log* is readily available for analysts using equipment that has a significant effect on the quality of test results. The records include (where applicable):

- Identity of the equipment
- Manufacturer name, type identification, model number, serial number, and asset number, if applicable
- Evidence of verification that equipment conforms with specified requirements
- Location of the equipment (room #), if appropriate
- Manufacturer's instructions, if available, or reference to their location
- Maintenance log, including any damage, malfunction, modification, or repair to the equipment
- Date permanently removed from service, if applicable

When equipment is retired, the records shall be maintained and available for at least one full accreditation cycle.

EQUIPMENT IDENTIFICATION

Each piece of equipment is labeled with a unique identifier[13]; those unique identifiers are recorded in the *Instrument Maintenance and Temperature Log* kept in each Forensic Serology laboratory area housing such equipment.

HANDLING AND MAINTENANCE OF EQUIPMENT

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding Handling and Maintenance of Equipment.

Maintenance of equipment having a significant impact on the quality of results is a planned activity. See TABLE 9 below. All equipment shall be checked for cleanliness annually.

[13] e.g. ALS-2, pH-1, WB-3

TABLE 9 Equipment Maintenance		
Equipment	**Maintenance Required**	**Frequency of Maintenance**
Water Bath	Clean interior of water bath (drain water bath, clean interior, refill with water to appropriate level, allow water to warm, check temperature)	Annually Document in maintenance log
Alternate Light Source	Clean alternate light source (clean exterior surfaces)	Annually Document in maintenance log
Centrifuge	Clean centrifuge (remove the spinning bowl and clean the interior of centrifuge, clean the spinning bowl, replace the spinning bowl, clean exterior surfaces)	Annually Document in maintenance log
Oven	Clean oven (clean interior and exterior surfaces)	Annually Document in maintenance log
View Box	Clean view box (clean exterior surfaces)	Annually Document in maintenance log
Refrigerator	Clean refrigerator (clean interior and exterior surfaces)	Annually Document in maintenance log
Microscope	Clean microscope (clean exterior surfaces)	Annually Document in maintenance log
pH Meter	Clean pH meter (clean exterior surfaces)	Annually Document in maintenance log
	Verify adequate electrode buffer covers probe tip when using & storing pH Meter in case. Replenish volume, if needed.	With each use of pH Meter Document in maintenance log
Ductless Hood	Change filters	As needed Document in maintenance log
	Clean hood (interior and exterior surfaces)	Annually Document in maintenance log

6.5 METROLOGICAL TRACEABILITY

The Serology Unit does not perform measurements that require metrological traceability.

6.6 EXTERNALLY-PROVIDED PRODUCTS AND SERVICES

6.6.1 GENERAL

See *ASCL Quality Manual* (ASCL-DOC-01).

6.6.2 RECORDS

If the Forensic Serology Unit transfers evidence to an outside laboratory[14], an *Inter-Laboratory Evidence Transfer Form* (ASCL-FORM-07) must be completed and entered into the case file. The Inter-Laboratory Evidence Form may be waived for items funded out of a grant and/or items under a contract. Any cost incurred by the laboratory must be approved by the Assistant Director.

All external laboratories performing casework or calibration for the Arkansas State Crime Laboratory must be accredited by an accrediting body recognized by the Arkansas State Crime Laboratory. These laboratories must provide the Arkansas State Crime Laboratory with documentation of accreditation, which is maintained in Qualtrax®.

6.6.3 COMMUNICATION

The Forensic Serology Unit will communicate its requirements (if any) to external providers for:

a) the products and services to be provided
b) the acceptance criteria
c) competence, including any required qualification of personnel
d) activities that the laboratory, or its customer, intends to perform at the external provider's premises

[14] For example: FBI, NMS, Bode Technologies

7 PROCESS REQUIREMENTS

7.1 REVIEW OF REQUESTS, TENDERS, AND CONTRACTS

7.1.1 GENERAL

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding Requests, Tenders, and Contracts.

DISCUSSION OF REQUESTS

Discussion with customers should take place when information is unclear as to:

- what type of analyses are needed
- where or from whom the item(s) of evidence originated
- if an arrest has been made under qualifying offenses (per current Arkansas Code)
- if elimination standards are required from any of the involved individuals
- if all appropriate types of analyses have been requested by the agency

REVIEW OF CONTRACT/REQUESTS

The Physical Evidence Section handles a large variety of cases including, but not limited to, rape, homicide, assault and battery, motor vehicle hit-and-run, property crimes, and arson. The *Case Management Guidelines* (ASCL-DOC-10) provides a general priority system for cases submitted to the Physical Evidence Section.

The Physical Evidence Section is composed of two units:

1) Serology Unit
2) Trace Evidence Unit

The Serology Unit receives evidence items associated with crimes that have been submitted to the ASCL for examination for the presence of blood, semen, saliva[15], and transfer or touch DNA.

The Trace Evidence Unit analyzes the following types of evidence: hairs, primer gunshot residue from suspects, and ignitable liquids.

The Section Chief or designee will review requests as cases are assigned to the Serology Unit.

SEND BACK LETTERS

Evidence is sometimes accepted that may appear to meet the laboratory's acceptance guidelines

[15] Serology doesn't identify saliva, but may collect samples of possible saliva (e.g., swab of bite marks, tape lift of a facemask, swab of toothbrush bristles) for the DNA potential that those stains may hold.

but upon further evaluation by the Physical Evidence Section is later determined to not be acceptable for analysis. Cases failing to meet the Touch DNA Policy as outlined in the *Case Management Guidelines* (ASCL-DOC-10) are placed in a RETURN file. Periodically, this file is distributed to an analyst or trainee to create a notification[16] stating that the case will not be analyzed and the evidence will be returned. This notification undergoes a technical and administrative review.

DOCUMENTATION OF COMMUNICATIONS

Communication between analysts and investigating officers resulting in obtainment of new information will be documented in an appropriate manner, such as an email, a conversation sheet (ASCL-FORM-06), or other appropriate document and will be stored in the case file.

REQUESTS FROM THE MEDICAL EXAMINER'S OFFICE

Requests originating from the Medical Examiner's office are handled exactly like requests from outside agencies. Reports are written, technically reviewed, and administratively reviewed. Medical Examiner's Office requests and Agency requests may be combined into one report.

7.1.2 INAPPROPRIATE REQUESTS

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding inappropriate requests.

7.1.3 STATEMENTS OF CONFORMITY

The ASCL does not issue reports containing statements of conformity.

7.1.4 RESOLUTION OF DIFFERENCES

Any difference between the request or tender and the contract shall be resolved before any work commences. Each contract shall be acceptable both to the ASCL and the customer.

7.1.5 DEVIATION FROM THE CONTRACT

When the customer agrees to the contract, the customer agrees that the ASCL may make deviations as deemed necessary. However, the customer will be notified (e.g., iResults, phone call, e-mail) if the Serology Unit goes outside its scope of testing.

7.1.6 AMENDMENT OF THE CONTRACT

If the contract needs to be amended after work has begun, the contract shall be reviewed (as stated above) by the discipline making the amendment, and all affected personnel shall be notified.

[16] Send Back Letter request on JusticeTrax®

7.1.7 COOPERATION WITH CUSTOMERS

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding cooperation with customers.

7.1.8 RECORDS OF REVIEW

See *ASCL Quality Manual* (ASCL-DOC-01) for information regarding records of review.

7.2 SELECTION, VERIFICATION, AND VALIDATION OF METHODS

7.2.1 SELECTION AND VERIFICATION OF METHODS

7.2.1.1 SELECTION OF METHODS

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding selection of methods.

7.2.1.1.1 TEST METHODS

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding test methods.

If it becomes necessary to deviate from a documented method and/or procedure, the deviation must be technically justified and authorized by the appropriate Section Chief. The deviation will be documented in the case record. Each Section Chief will keep a log of method/procedure deviations.

7.2.1.1.2 COMPARISON OF KNOWNS AND UNKNOWNS

The Serology Unit does not use any methods that involve the comparison of knowns[17] and unknowns.

7.2.1.1.3 CALIBRATION METHOD SELECTION

The ASCL does not perform calibration.

7.2.1.2 METHOD AVAILABILITY

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding method availability.

7.2.1.3 METHOD VERSION

Only the current approved version of a method may be used in casework.

[17] Knowns (e.g. known blood sample) submitted in cases worked by analysts in the Serology Unit are documented and repackaged to be sent to the Forensic DNA section. No comparison activities are conducted with these samples by the Serology Unit.

7.2.1.4 METHOD SELECTION

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding method selection.

The Serology Unit uses appropriate methods for all testing and evidence handling. The methods listed in section 9 encompass the most commonly encountered evidence types and are intended to serve as guidelines to analysts. Analysts have discretion in choosing appropriate procedure(s) for a particular piece of evidence, based on their training and experience.

7.2.1.5 METHOD VERIFICATION

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding method verification.

METHOD REVISION

If a method used in the Serology Unit is revised, the method verification must be repeated to the extent necessary to demonstrate that the method is still fit for service.

7.2.1.6 METHOD DEVELOPMENT

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding method development.

7.2.1.7 DEVIATION FROM METHOD

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding deviation(s) from method(s).

Unforeseen circumstances may arise which require deviations from the policies and procedures of the *Forensic Serology Quality Manual* (SER-DOC-01). In such situations, the request of exceptions to policy will be submitted in writing to the Physical Evidence Section Chief, or designee[18]. The request must include an adequate description of the circumstances requiring the action, a statement of the proposed alternative policy and procedure, and the intended duration of the exception. The Physical Evidence Section Chief will maintain documentation of the approved policy exception.

7.2.2 VALIDATION OF METHODS

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding:

- validation of methods
  - extent of validation
- validation procedure
- changes to validated methods

[18] This may be achieved via email message or documented contact form.

- relevance to needs
- validation records
- validation review and approval

7.3 SAMPLING

7.3.1 GENERAL

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding sampling.

7.3.2 SAMPLING METHOD

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding sampling method.

The sampling used by the Serology Unit is nonstatistical sampling. This type of sampling is a practice of selecting items to test or portions of items to test, based on training, experience, and competence. Nonstatistical sampling answers questions only about the portion tested. There is no assumption of homogeneity of the whole[19].

Example: Pair of pants with four stains—one stain is chosen to be tested based on the analyst's training and experience.

See sections 9.1.1, 9.4.3.2, and 9.5.3.2 for specific information on sampling method policies and procedures used by the Serology Unit.

7.3.3 SAMPLING RECORDS

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding sampling records.

See sections 9.4.3.3 and 9.5.3.3 for sampling records policies used by the Serology Unit.

7.4 HANDLING OF TEST ITEMS

7.4.1 GENERAL

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding the handling of test items.

[19] i.e., if a stain is tested and identified to be blood, the analyst does not assume that every stain on the garment is also blood.

7.4.1.1 HANDLING PROCEDURES

7.4.1.1.1 STORAGE

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding handling procedures/storage.

SECURING EVIDENCE

All evidence not in the process of examination/analysis shall be maintained in a secure, limited-access storage area under proper seal.

Each Serology Unit work area is equipped with lockable cabinets where evidence can be stored. Items too large for the work area cabinets may be placed under the analyst's bay area in sealed condition, in a scrape-down room, or other locked room for overnight or other long-term storage.[20]

UNATTENDED EVIDENCE

Evidence in the process of examination may be left unattended for a reasonable period of time in a secure limited-access area. The analyst shall take reasonable precautions to protect the evidence from loss, cross-transfer, contamination, and deleterious change.

7.4.1.1.2 PACKAGING AND SEALING

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding packaging and sealing.

EVIDENCE IN THE PROCESS OF EXAMINATION

Items with an expectation of frequent analysis may be considered "evidence in the process of examination/analysis" and may be stored unsealed in a limited access area as long as the evidence is protected from loss, cross-transfer, contamination, and deleterious change. Cases no longer in the process of examination shall be closed and the evidence properly sealed until analysis resumes or a new service request is received.

Evidence collected from a crime scene by laboratory personnel while assisting law enforcement shall be protected from loss, cross transfer, contamination, and deleterious change during transportation to the ASCL, whether in a sealed or unsealed container. Where appropriate, further processing to preserve, evaluate, document, or render evidence safe shall be accomplished prior to final packaging. The evidence shall be appropriately identified, packaged, and entered into the LIMS as soon as practical[21].

[20] The Serology Unit, as a whole, is a limited access area which requires proper authorization by administration as well as a key fob to enter. Items within this locked laboratory area are considered secured.
[21] While not mandatory, Serology Unit analysts assisting in the collection of evidence from a crime scene are encouraged to transfer any collected/packaged evidence to the investigating agency while still on-site at the

EVIDENCE RETENTION

Samples (e.g., cuttings, swabs, DNA extraction tubes, and tape lifts) retained for analysis by the DNA Section will be placed in the locked cabinet labeled "FD in PE Secure Storage" or "FD Secure Storage."

Samples (e.g., hairs and tape lifts) retained for analysis by the TRACE Unit or the DNA Section will be placed in the locked cabinet labeled "PE Secure Storage."

Samples retained for potential future analysis will be placed in the locked "Long Term Storage" area.

Samples collected and retained (e.g., tape lifts, hairs) are periodically returned to the agency. Items are usually retained for at least 5 years.

Storage locations for all retained evidence will be recorded in JusticeTrax®.

7.4.1.1.3 CHAIN OF CUSTODY

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding chain of custody.

7.4.1.1.4 CUSTOMER NOTIFICATION

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding customer notification.

7.4.2 ITEM IDENTIFICATION

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding item identification.

The Serology Unit employs the use of Q and K numbers in identification of test items and known samples[22], respectively. Questioned (or test) items, those items examined for the presence of body fluids, are labeled with Q numbers, (i.e., Q1, Q2). Known samples, (e.g., known blood samples or known oral swabs or known hair samples) are labeled with K numbers, (i.e., K1, K2).

Samples retained from questioned items are further labeled with sub-item identifiers that designate the type of sample retained, (e.g., Q#-1S, Q#-1B, Q#-1T, Q#-C where "S" indicates a stain initially identified for semen testing, "B" indicates a stain initially identified for blood testing, "T" indicates an area initially identified as a potential transfer or touch DNA sample, and "C" indicates a

scene. This allows the agency to then formally submit the evidence (along with any additional items they may have collected) to the Arkansas State Crime Laboratory.

[22] Known samples are also referred to as exemplars by some members of the forensic science community.

substrate control sample). However, a sample identified as Q#-1S may also be tested for blood and conversely, a sample identified as Q#-1B may be tested for semen.[23]

If an item of evidence is being retained solely for the purpose of examination by another section, it is equally acceptable to identify the sample by the evidence number that the submitting agency used. No serological testing is typically conducted on these types of samples[24] and therefore, a Q# or K# is optional, but not necessary.

7.4.2.1 EXTENT

All items received by the Serology Unit will be identified as detailed in section 7.4.2.

7.4.3 DEVIATIONS

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding deviations (packaging).

If the analyst discovers a significant inconsistency[25] between the stated and actual contents of a package, or if there is doubt about the suitability of an evidence item for testing, then the analyst shall attempt to contact the customer before proceeding. All contacts will be documented in the case record (e.g., using an *Agency Contact Form* (ASCL-FORM-06) or by email). For minor inconsistencies, the analyst shall use their judgement on whether to contact the customer, but must make a note of the discrepancy in the case file.

If the customer requires testing, acknowledging a deviation from specified conditions, a disclaimer will be included in the report indicating which results may be affected by the deviation.

7.4.4 ENVIRONMENTAL CONDITIONS

Occasionally, evidence will be submitted to the laboratory that requires refrigeration[26]. Until the evidence is received by the analyst assigned to the case, it is stored in an Evidence Receiving Section refrigerator (usually specified by an IB# on the submission form). Once the analyst receives the evidence, they should transport it to their laboratory area and store it in a Serology Unit refrigerator until it can be processed.

[23] Slight variation among analysts in the style they choose to label sub-item stains and samples (within reason) is expected. As long as it is clear as to what type(s) of testing was/were conducted on which specific stains, this is acceptable.
[24] e.g., hairs collected from a scene or person by a submitting agency
[25] A significant inconsistency raises doubt as to the identity of a submitted item, not solely the accuracy of its description. For example, blue plants described as a red shirt.
[26] Refrigeration is recommended in situations where decomposition or bacterial/fungal growth has begun or is a concern. Sexual assault kits containing liquid blood and urine tubes can be negatively affected by non-refrigerated temperatures.

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding environmental conditions.

7.5 TECHNICAL RECORDS

7.5.1 GENERAL

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding technical records.

RECORDING DATES OF ANALYSES

The date that a case is started shall be recorded at the beginning of the notes or on the case worksheet. Dates of analysis are documented in the notes. The ending date for work is considered to be the date recorded in JusticeTrax® as "Draft Complete."

DATA RECORDING

Observations, test results, and other data are recorded at the time they are made and shall be identifiable to the specific task.

7.5.1.1 TECHNICAL RECORD RETENTION

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding technical record retention.

EXAMINATION RECORDS

Examination records are any records generated by the analyst/examiner for a case file (e.g., notes, worksheets, photographs, spectra, printouts, charts, and other data). Examination records that are essential for the evaluation and interpretation of the data must be stored in the appropriate folder within the "Request" folder in the LIMS case file. If some records[27] are not feasible to incorporate the examination records in the LIMS case file, these records may be stored external to the LIMS case file. The location of these records must be specified in the case file.

EXAMINATION RECORD DOCUMENTATION

The unique Arkansas State Crime Laboratory case number (YYYY-000000) (handwritten or electronically generated) and the analyst's handwritten initials or secure electronic equivalent of initials or signature must be on all examination records in the case file.

[27] e.g., large packet of crime scene photos submitted by law enforcement may need to be stored in a sealed manila envelope in PE Secure Storage.

RECORD PREPARATION

When an individual other than the issuing examiner prepares examination records, the initials of that individual(s) shall be on the page(s) of examination records representing their work[28]. It should be clear from the case record which analyst performed all stages of the examination/analysis[29].

ADMINISTRATIVE RECORDS

All other records contained in the case file (e.g., conversation records, email messages, case review forms) will be considered administrative records and will normally be stored in the "Case Attachments" folder in the LIMS case file. It is acceptable to place an administrative memorandum in a "Request" folder after the draft complete milestone if (and only if) it does not serve as an examination record (i.e., it solely helps explain the administrative information contained within the examination record).

Conversation records, including emails, shall be stored in the "Case Attachments" folder. It is also recommended to store a copy in the appropriate Serology Request folder in the LIMS case file.

ADMINISTRATIVE RECORD DOCUMENTATION

The unique Arkansas State Crime Laboratory (ASCL) case number (YYYY-000000) (handwritten or electronically generated) must be on all administrative records in the case file.

Analysts are encouraged to hand-write the case number and their handwritten initials on pages that are scanned in which do not already have this information in the header. If the page quantity is lengthy, (i.e., HIPAA protected medical records, Incident Reports) handwriting this information on the first page of the set, at a minimum, is encouraged.

7.5.1.2 ABBREVIATIONS

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding abbreviations.

A master abbreviation list for the Serology Unit is located in Qualtrax®. See *Master Abbreviation List* (SER-DOC-03).

[28] If a trainee's initials appear at the bottom of an examination page, it is to be understood that all examination records were documented by the trainee.

[29] This includes supervised benchwork (or examinations) performed by a trainee. It should be clear what examination/analyses the trainee performed and what examination/analyses the qualified analyst performed. This can be done by (a) recording a statement at the beginning/end (or where appropriate) of the examination notes that indicates who (analyst vs trainee) performed which testing; or (b) adding the trainee's initials at each test or test item on which they performed analysis (it is understood that if no initials are present, the examination/analysis was performed by the case analyst).

7.5.1.3 TECHNICAL RECORD SUFFICIENCY

Technical records to support a report[30] shall be such that, in the absence of the analyst, another competent reviewer could evaluate what was done and interpret the data. See the "Notes/Documentation Requirements" section under each Test Method of section 9 for the required record documentation.

7.5.1.4 TECHNICAL RECORD PERMANENCY

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding technical record permanency.

7.5.1.5 REJECTION

If data, an observation, or a calculation is rejected, the following information will be recorded in the technical record:

- The reason for the rejection
- The identity of the person rejecting
- The date of the rejection

7.5.1.6 CALIBRATION DATA

The Serology Unit does not use equipment that is calibrated and therefore, has no calibration data to document.

7.5.2 AMENDMENTS TO TECHNICAL RECORDS

Amendments[31] to technical records must be trackable to previous versions or to original observations. Both the original and amended data/files will be retained, including:

- The date of alteration
- An indication of the altered aspect(s)
- The personnel who made the alteration(s)

DOCUMENTATION OF CORRECTIONS

Any corrections made to existing hardcopy technical records will be made by an initialed and dated single strikeout (so that what is stricken can still be read) by the person making the change. All additions will be initialed and dated. Correction fluid or correction tape may not be used.

[30] Including results, opinions, and interpretations
[31] Including additions, deletions, changes, interlineations, or any other modification to the original information

Changes made to electronic documents must allow the reviewer to track what changes were made to the document, who made the change, and when. If a correction is made, the original version will be maintained[32].

Contemporaneous[33] revisions to technical records are not considered to be amendments.

When the analyst/examiner has completed a request, then they will set the milestone(s) in JusticeTrax® to draft complete. Examination records for a request will be considered completed at this time. If a change is subsequently made to the examination record, the original record will remain in the electronic case file and the changed record will be stored with a different name (e.g., amended notes). There shall be sufficient information to determine what was changed.

If a report is changed after it has been draft completed, but before release, the original version will be maintained in the case record[34].

7.6 EVALUATION OF MEASUREMENT UNCERTAINTY

The Serology Unit does not perform any tests that require the evaluation of measurement uncertainty.

7.7 ENSURING THE VALIDITY OF RESULTS

7.7.1 GENERAL

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding ensuring the validity of results.

The Serology Unit of the Physical Evidence Section maintains this quality manual, which contains quality control procedures and continually monitors and ensures the validity of test results.

CONTROLS AND STANDARDS

Documentation of controls and standards employed by the Serology Unit are described in this manual within the Test Methods (section 9).

QUALITY CONTROL DATA

Methods concerning nonconformity of testing in control/standard data are described in this manual within the Test Methods (section 9).

[32] A second copy of the document is not necessary if it has not yet been placed into JusticeTrax®; the correction can be made on the original notes.
[33] Contemporaneous means at the same time. **Amendments made after moving on to the next item/task are not considered to be contemporaneous.**
[34] Index into JusticeTrax® Case Attachments with the name DRAFT REPORT.

7.7.1.1 VERIFICATION

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding verification.

DOCUMENTING VERIFICATIONS

Verification is an independent examination of the evidence by another competent analyst to confirm the primary analyst's conclusions. Verifications shall be performed by another analyst qualified in the same discipline/sub-discipline. Verifications must be documented in the case file indicating that the critical finding has been verified and agreed to, by whom, and when the verification was performed.

If the individual performing the verification draws a different conclusion from the primary analyst, the issue shall be brought to the attention of the Section Chief for resolution. The final conclusion shall be verified and documented as described above.

The only required verification conducted in the Serology Unit is when spermatozoa cells are identified by an analyst. Identification of spermatozoa cells shall be verified by another qualified analyst. If the confirming analyst draws the same conclusion as the primary analyst, documentation will be made on the worksheet with the initials of the confirming analyst. If the verification is conducted on a date different from the date at the top of the worksheet, the confirming analyst will document the date near their initials or at the top of the appropriate column[35] of the appropriate semen examination worksheet. (See also section 9 Test Methods)

7.7.1.2 CASE REVIEW

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding case review.

All cases resulting in a Laboratory Report will be technically and administratively reviewed prior to the release of the report. The review process must confirm that electronic versions of all necessary documentation are in the imaging module of LIMS. In the Serology Unit, case review documentation shall be recorded on a case review form[36].

If a reviewer discovers an error in the case record, the reviewer must document the error (using a case review form) and inform the analyst. If the analyst and the reviewer cannot reach consensus, then both the analyst and reviewer must meet with the Section Chief (or designee) for resolution.

All non-conforming work identified during review will be handled according to section 8.7 (Corrective Action).

The successful completion of technical and administrative review is recorded by the setting of the appropriate milestone(s) in JusticeTrax®. (See also section 7.5.1.5)

[35] "Witness" column
[36] The *ASCL Case Review Form* (ASCL-FORM-05), or a discipline-specific form

7.7.1.2.1 TECHNICAL REVIEW

The technical review will include a thorough review of the analyst's examination records to ensure that the records support the reported results.

At a minimum, the technical review shall include a review of all examination records and the report to ensure that:

- All necessary analyses are performed and documented according to established guidelines
- The case data supports the results and/or conclusions in the report
- The report is accurate
- Associations and results are properly qualified in the report
- The report contains all required information

The technical review includes, but is not necessarily limited to: bench notes, spectra, graphs, external telephone conversation records, investigative reports, sketches, diagrams, and laboratory reports. The records must provide an adequate basis for any reported conclusions.

The technical review does not shift the responsibility for the forensic findings to the reviewer, but the reviewer has the responsibility of ensuring that the case record provides an adequate basis for the conclusion.

It is the responsibility of the technical reviewer to report serious or repetitive deficiencies to the Section Chief. If the technical reviewer discovers a problem that raises an immediate concern regarding the overall quality of the analyst's work, the technical reviewer must promptly notify the Section Chief. The Section Chief will consult with the Quality Assurance Manager and Assistant Director to determine whether a Quality Assurance Concern is warranted.

Technical reviews must be conducted by individuals competency-tested and authorized by the appropriate Section Chief to perform the testing work that is being reviewed[37]. This authorization shall be documented on the *Analyst & Technician Competency Authorization Documentation* form (ASCL-FORM-62).

An individual conducting technical review does not have to be an active examiner or undergo proficiency testing. The reviewer must have sufficient knowledge of the discipline to verify compliance with the laboratory's technical procedures and that the reported conclusions are supported by the examination documentation. For those individuals not currently competent in the reviewed discipline, the Section Chief shall write an authorization memo/letter which will be maintained in Qualtrax®. Technical review of an examination record or report shall not be conducted by the author or co-author.

Verification of a critical finding does not constitute authorship, and does not disqualify the verifier from performing technical review.

[37] The technical reviewer need not be an ASCL employee, currently proficiency tested, or currently performing the work

All cases must be TECHNICALLY and ADMINISTRATIVELY reviewed. After a report has been written, the header of the *PE Review Form* (SER-FORM-07) will be completed by the analyst. This form will be placed in the location established for TECHNICAL review. Upon completion of this review, the form will be placed in the location established for ADMINISTRATIVE review. Once the administrative review is complete, the review form will be scanned into Case Attachments in JusticeTrax®.

DOCUMENTATION

If a reviewer discovers an error in the case record, the reviewer must document the error[38] on the *PE Review Form* (SER-FORM-07) and inform the analyst. If the analyst and reviewer cannot reach a consensus, then both the analyst and reviewer must meet with the Section Chief (or designee) for resolution.

If any corrective actions were made on the review sheet, the ADMINISTRATIVE reviewer will return the sheet to the Section Chief upon completion of the administrative review.

7.7.1.2.2 ADMINISTRATIVE REVIEW

Administrative review includes a review of spelling and grammar, markings[39], descriptions of evidence and seals, and other appropriate documentation.

Administrative review may be conducted by any individual qualified to perform technical review. Administrative review shall not be conducted by the author of the report.

At a minimum, the administrative review shall include:

- A review of the report to ensure consistency with laboratory policy and editorial correctness
- A review of all administrative and examination records to ensure that they contain the unique ASCL case number and are stored properly in LIMS
- A review of the examination records to ensure dates are recorded to indicate when the work was performed, and
- A review of examination records to ensure that all corrections in the case file are made consistent with laboratory policy

7.7.1.2.3 TESTIMONY REVIEW

Testimony must also be technically reviewed by a competency-tested and authorized reviewer. This can be achieved in a couple of ways, including:

- Direct observation of the testimony
- Review of transcripts of testimony given by an examiner

[38] Include the reason for the rejection, the identity of the reviewer rejecting the case record and the date of the rejection.
[39] For example, case number, date, and initials on appropriate pages

Testimony review of each testifying analyst or examiner shall occur at least once per accreditation cycle, when practicable. If this review is not practicable, a memorandum will be generated detailing the reason(s). This documentation will be maintained in Qualtrax® on the Personnel tab.

The Physical Evidence Section Chief reserves the right to increase the frequency of testimony review for any reason. An example would be if the analyst being reviewed is found to have had any serious or repetitive deficiencies in the technical aspects of testimony and/or whose testimony has raised an immediate concern regarding the overall quality of the analyst's work.

TESTIMONY REVIEW SCHEDULE

- First testimony as a new analyst- Reviewed by PE Section Chief (or Serology Quality Manager), if possible.
- One testimony review per testifying analyst per accreditation cycle (4 years.)

The requirements for technical review of testimony for Serology Unit cases are as follows:

- The person conducting the technical review of testimony must be competent to do so, which shall be documented on the *Analyst & Technician Competency Authorization Documentation* (ASCL-FORM-62) form.
- The reviewer shall physically observe[40] (or review the transcripts of testimony of) the analyst testifying.
- A *Testimony Evaluation Form* (ASCL-FORM-04) will be completed by the reviewer, and signed by both the analyst and their supervisor. Feedback shall be given, both positive and in any area needing improvement. If the evaluation is less than satisfactory, the Section Chief will determine whether remedial actions are required, which may include the following:
  - Re-training, including a mock trial
  - Courtroom monitoring by the Section Chief for a designated period of time

  Feedback on testimony may also be solicited from court (or other) personnel using a *Testimony Evaluation Form*, but this feedback does not meet the testimony review requirements of this section.

7.7.2 INTERLABORATORY COMPARISONS

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding interlaboratory comparisons.

[40] In courtroom-attended proceedings or via observation of the analyst participating in video testimony (e.g., GoToMeeting™)

7.7.2.1 EXTERNAL PROFICIENCY TESTING

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding external proficiency testing.

The Serology Unit will successfully complete at least one external proficiency test annually.

7.7.3 MONITORING ACTIVITY ANALYSIS

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding monitoring activity analysis.

7.7.4 INDIVIDUAL PERFORMANCE MONITORING

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding individual performance monitoring.

BODY FLUID ID

Each analyst and technical support personnel engaged in testing activities, verifications, case review, or the authorization of results in the Serology Unit shall successfully complete at least one Body Fluid Identification proficiency test per year, at minimum.

Interlaboratory[41] proficiency tests will be obtained from a provider that meets the requirements of ISO/IEC 17043 unless special circumstances arise and an internal test is required or warranted.

Internal proficiency tests may include previous external proficiency samples, samples retained from casework, re-examination techniques, or blind techniques.

7.7.5 PERFORMANCE MONITORING REQUIREMENTS

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding performance monitoring requirements.

CRITERIA FOR SUCCESSFUL COMPLETION OF PROFICIENCY TESTING

BODY FLUID ID

All body fluids[42] are correctly identified on the provided proficiency test evidence using the appropriate test methods (See section 9). The Physical Evidence Section Chief is responsible for comparing the analytical results to the expected results, determining if the analytical results are acceptable, and for reviewing these results with the analyst.

[41] Previously called external proficiency tests
[42] Blood and semen are the only body fluids currently tested for at the ASCL

Notes are properly documented (See section 9 documentation procedures). Qualified analysts will conduct technical and administrative reviews of proficiency tests.

Appropriate case file components[43] are recorded in JusticeTrax® (See section 9 for JusticeTrax® procedures).

Appropriate areas of the interlaboratory proficiency test provider electronic forms, if applicable, are completed and electronically sent to the appropriate party: (a) the Proficiency Test Provider; or (b) the corresponding DNA Analyst assigned to a shared proficiency test.[44]

7.7.6 PERFORMANCE MONITORING SCHEDULE

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding performance monitoring schedule.

The Serology Unit maintains a documented schedule of proficiency testing on the S: drive. (See "Serology Proficiency Testing Schedule" under the "ISO Documents-Serology" folder)
The Serology Unit will participate in at least one interlaboratory[45] Body Fluid ID proficiency test per calendar year.

Each individual engaged in testing activities (both analysts and technical support personnel) shall be proficiency tested annually in each discipline in which they perform testing.

Only personnel already competent in the covered testing may be assigned to or participate in an external proficiency test. Each laboratory discipline shall have a documented plan for proficiency testing designed to meet all requirements.

7.7.7 PROFICIENCY TEST SOURCING

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding proficiency test sourcing.

7.7.8 PERFORMANCE MONITORING RECORDS

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding performance monitoring records.

Current proficiency test information is maintained using a Qualtrax® workflow.

Additionally, the JusticeTrax® case file will contain:

[43] Itemizations, indexed/scanned notes, DNA submissions, report at draft complete milestone, etc.
[44] Intralaboratory proficiency tests will not routinely be assigned to a DNA analyst for completion.
[45] Formerly called an external proficiency test

- All administrative and examination documentation
- Quality Assurance Concern documentation, when applicable

7.8 LANGUAGE FOR REPORTS AND TESTIMONY

7.8.1 GENERAL

7.8.1.1 REVIEW AND AUTHORIZATION OF RESULTS

All results will be reviewed and authorized before release[46].

7.8.1.1.1 DOCUMENTATION

Both the review of results and the authorization of results are performed by the author of the report, and are documented by the setting of the draft complete milestone.

ASCL analysts issuing a report based on examination records generated by another individual shall complete and document a review of all relevant pages of documentation in the case record (e.g., initialing each page of the examination record, the use of a review checklist or statement).

ASCL analysts offering testimony based on examination records generated by another individual shall complete a *Court Case Review Form* (ASCL-FORM-57) before testifying.

7.8.1.2 REPORTS

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding reports.

7.8.1.2.1 REPORT DISTRIBUTION

Reports are normally made available to the customer electronically through JusticeTrax® iResults. Facsimile or email may be used to transmit results to the customer, but the sender must follow the requirements of A. C. A. § 12-12-312 and the policy on Confidentiality of Records (section 4.13.1.3).

7.8.1.2.2 REPORTING PROCEDURE

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding reporting procedure. The current Serology Autotext document is maintained on the S: drive and is used as a model for the Serology report writing process in JusticeTrax® LIMS. It contains all of the common wording options for Serology reports. Wording is selected by the analyst depending on the case/analysis type. This wording is intended to meet the needs of the vast majority of cases encountered. However, wording may be tailored by the analyst as needed when non-routine or unique

[46] Communicating (+) spermatozoa cell results to the customer *after verification* is the only exception to this policy (See section 7.7.1.1).

information also needs to be reported. The Physical Evidence Section Chief may be consulted to aid in determining this wording, if needed.

REPORT ELEMENTS

Serology Reports shall include the following:

- Identification of what was tested (serologically)
- The results of those tests (applicable blood and semen testing)
- If no hairs were recovered from the pubic hair combings envelope
- Identification of what was not examined (also submitted, not examined list)
- Any DNA reference samples that may be needed
- A list of any retained items, cuttings from items, tape lifts from items, swabs from items, and/or Hair(s)/Fiber(s) from items
- Communicates to the customer that future DNA analysis will occur, if applicable.

The current text options[47] used for the Serology Report "Further Explanation of Results" section are stored on the S: drive as "Serology Autotext."

For items received, but not examined, those items shall be addressed in the body of the report (i.e., under Further Explanations of Results- "Also submitted, but not examined: E-1, E-3, and E-4a").

See section 9 Report Writing subheadings for report procedure information specific to the different Test Methods used in the Serology Unit.

7.8.1.2.3 CALIBRATION

The ASCL does not perform calibration or issue calibration reports.

7.8.1.3 SIMPLIFIED REPORTING

The ASCL, in agreement with its customers, reports in a simplified way. This agreement is documented on the submission form by the customer's signature.

7.8.1.3.1 REPORT ELEMENTS

A list of the specific report elements included and excluded on reports is available to the customer on the ASCL website. A link to where this list is located on the website is included on the *Evidence Submission Form* (ASCL-FORM-12 or ASCL-FORM-63). All elements are documented (when applicable) and available upon customer request.

[47] This autotext is intended as a general template for report writing with the understanding that some modifications to wording and/or content are acceptable. Using training and experience, the analyst will choose which portion of the autotext best reflects their case.

7.8.2 COMMON REQUIREMENTS FOR REPORTS

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding common requirements for reports.

7.8.3 SPECIFIC REQUIREMENTS FOR TEST REPORTS

7.8.3.1 ADDITIONAL STATEMENTS

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding additional statements.

7.8.3.2 REPORTING SAMPLING

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding reporting sampling.

The Serology Unit does not report the nonstatistical sampling used in test methods pertaining to body fluid identification.

Certain information is to be included in the examination notes regarding samples collected and retained:

- Unambiguous identification of the item sampled/retained
- When applicable, the location of sampling, which may include a written description of the location, diagrams, sketches, or photographs.
- Description of size of stains that are being retained. Approximate measurements may be used, (i.e., ~ ½ inch diameter, ~ 1”×1”); descriptive measurements may also be used, (i.e., stain covers entire crotch, ~80% of garment was covered). Photographs may also be used.

Any samples retained from an item of evidence will be indicated on the report. See applicable Serology Report Writing headings within section 9 Serology Unit Test Methods.

7.8.4 SPECIFIC REQUIREMENTS FOR CALIBRATION CERTIFICATES

The ASCL does not perform calibration or issue calibration certificates.

7.8.5 REPORTING SAMPLING—SPECIFIC REQUIREMENTS

See above (section 7.8.3.2.)

7.8.6 REPORTING STATEMENTS OF CONFORMITY

The ASCL does not issue statements of conformity.

7.8.7 REPORTING OPINIONS AND INTERPRETATIONS

7.8.7.1 AUTHORIZATION

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding authorization.

7.8.7.2 SCOPE OF OPINIONS/INTERPRETATIONS

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding scope of opinions/interpretations.

7.8.7.3 DIALOGUE

When opinions or interpretations are directly communicated by dialogue to a customer, a record of the communication will be retained[48]in the case record.

7.8.8 AMENDMENTS TO REPORTS

7.8.8.1 IDENTIFYING THE CHANGE(S)

An amended report is necessary if an error is found on an issued report (including reports uploaded to iResults). An "amended request" will be created in the LIMS and all administrative and examination records for the amended analysis will be added to the electronic case record. Administrative and technical reviews are required before an amended report is issued. When an amended report is necessitated by a change in analytical results, then the Section Chief or Section Quality Manager will perform the technical review on the amended request. Documentation of this review will be incorporated into the original case file.

The original report and all original records will be kept in the case record.

An amended report is generally not needed when an agency revises or corrects administrative information that they provided at the time of submission[49] after a report has already been issued. Exceptions can be made for individual cases, when appropriate.

The amended report is intended to replace the original report, and will contain all of the unchanged results from the original report, as well as the newly-amended results. Any change of information will be clearly identified. Where appropriate, the reason for the change will be included in the report[50].

[48] For example, using an *Agency Contact Form* (ASCL-FORM-06)
[49] For example, correcting the spelling of a name, or changing an incorrect agency case number
[50] For example, "The blood test result for Q# was changed from (Negative) to (Identified) due to a typographical error."

The disclaimer will normally be contained in a note at the bottom of the report, but may be alternately listed in the result text if this makes the reason for the amendment clearer to the customer.

7.8.8.2 STYLE OF AMENDMENT

Any amendments to an issued report are made by issuing a complete new report.

7.8.8.3 IDENTIFYING THE AMENDED REPORT

The statement "*AMENDED REPORT TO ORIGINAL [TYPE] REPORT ON [DATE]*" (or equivalent) will appear below the header information and above the listing of the evidence and the results[51]. The amended report will contain all of the items on the original report and any amendments.

The original report must be stored in the JusticeTrax® case record.

7.8.9 SUPPLEMENTAL REPORTS

A supplemental report is necessary when additional evidence is received after the original report has been issued, additional requests for analysis are made, or other additional testing is required in a case[52]. A "supplemental request" will be created in the LIMS, and all administrative and examination records for the additional evidence will be added to the electronic case record. Administrative and technical reviews are required before a supplemental report is issued. The statement "SUPPLEMENTAL REPORT TO ORIGINAL [TYPE] REPORT ON [DATE]" (or equivalent) will appear below the header information and above the listing of the evidence and the results[53]. The supplemental report will contain the updated information from the additional analysis.

All original records will remain in the case record.

7.8.10 REPORTING GUIDELINES

- For reporting guidelines for specific test methods, refer to Section 9 of this manual.
- For each item of evidence analyzed, an analyst shall include the results and the conclusions drawn from confirmatory serological tests and examinations in a laboratory report when:
  - the presumptive serological test gives a positive result and further confirmatory testing is completed, also yielding a positive result. An analyst shall conclude blood or semen is identified (positive).

[51] The date of the original report must be entered in the "additional data" tab of the amended request.
[52] When additional evidence is received on a case that has not been completed, the additional evidence may be analyzed and included in the original report
[53] The date of the original report must be entered in the "additional data" tab of the supplemental request

- the presumptive serological test gives a positive result and further confirmatory testing is completed, yielding a negative result. An analyst shall conclude that blood or semen is not identified (negative).
- presumptive testing is not performed. For some confirmatory tests, prior presumptive testing is not required. When the test gives a positive result, an analyst shall conclude blood or semen is identified (positive). When the test gives a negative result, an analyst shall conclude that blood or semen is not identified (negative).

- For each item of evidence analyzed, an analyst shall include the results and the conclusions drawn from presumptive serological tests and examinations in a laboratory report when:
  - the presumptive serological test gives a negative result. An analyst shall conclude that blood or semen is not identified (negative).
  - the presumptive serological test gives a positive result but an insufficient quantity of sample remains for confirmatory testing. An analyst shall conclude that presumptive tests were positive so blood or semen is indicated; however, confirmatory tests were not conducted due to limited sample quantity. An analyst shall *not* state that a body fluid is identified.

7.8.11 TESTIMONY GUIDELINES

- 'Identification' for the presence of blood or semen when using a confirmatory serological test or examination is an analyst's conclusion that blood or semen was detected in a tested sample.
  - The basis for an analyst's conclusion that blood was 'identified' in a tested sample is the interpretation of a positive result from an appropriate confirmatory blood test.
  - The basis for an analyst's conclusion that semen was 'identified' in an examined sample is the interpretation of a positive result from an appropriate confirmatory semen test.
- 'Indicated' for the presence of blood or semen when using a presumptive serological test is an analyst's conclusion that blood or semen may be present in a tested sample.
  - The basis for an 'indicated' conclusion is the interpretation of a positive result from an appropriate presumptive serological test. A presumptive positive serological test result for blood or semen does not confirm the presence of either substance.
- 'Negative' for the presence of blood or semen when using a confirmatory serological test or examination is an analyst's conclusion that blood or semen could not be confirmed in a tested sample.
  - The basis for a 'negative' conclusion is the interpretation of a negative result from an appropriate confirmatory serological test or examination for blood or semen. Insufficient quantity and/or quality of biological material may affect the ability to detect the presence of blood or semen in a tested or examined sample.
- 'Negative' for the presence of blood or semen when using a presumptive serological test is an analyst's conclusion that no blood or semen was detected in a tested sample.
  - The basis for a 'negative' conclusion is the interpretation of a negative result from an appropriate presumptive serological test. Insufficient quantity and/or quality of biological

material may affect the ability of a presumptive serological test to detect the presence of blood or semen in a tested sample.

- If a bodily fluid is identified in casework evidence, an analyst shall make no assumption or suggestion as to how that substance was transferred to the evidence.
- If a bodily fluid is identified in casework evidence, an analyst shall make no assumption or suggestion as to how long that substance has been present in the evidence.
  - In the rare occasion that a body cavity swab is tested, resulting in identification of semen, an analyst may provide a *general* timeframe of persistence of semen in a particular body cavity.
- If a presumptive serological test gives a result that is interpreted as an 'indication,' but a confirmatory serological test or examination conducted on the same sample gives a result that is interpreted as an 'identification,' an examiner may assert that blood or semen was 'identified' in the tested or examined sample.
- If a presumptive serological test gives a result that is interpreted as an 'indication,' but a confirmatory serological test or examination conducted on the same sample gives a result that is interpreted as a 'negative,' an analyst may assert that blood or semen is 'indicated' in the tested sample.
- An analyst may assert that presumptive serological tests may yield false positive results due to the specificity of such tests.
- An analyst may assert that confirmatory serological tests may yield false negative results due to the sensitivity of such tests.
- When analyzing a portion of an item, an analyst shall not state their conclusion applies to the entire item or group of items.
- An analyst may assert that an insufficient quantity or quality of blood or semen can limit the ability of both presumptive and confirmatory serological tests to detect those substances.
- An analyst shall not assert that presumptive or confirmatory serological tests or examinations are infallible or have a zero error rate.
- An analyst shall not provide a conclusion that includes a statistic or numerical degree of probability except when based on relevant and appropriate data.
- An analyst shall not cite the number of forensic serological tests or cases worked in his or her career as a direct measure for the accuracy of a proffered conclusion. An analyst may cite the number of forensic serological tests or cases worked for the purpose of establishing, defending, or describing his or her qualifications or experience.
- An analyst shall not use the expressions 'reasonable degree of scientific certainty,' 'reasonable scientific certainty,' or similar assertions of reasonable certainty in either reports or testimony.

7.9 COMPLAINTS

7.9.1 GENERAL

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding complaints.

The ASCL processes all complaints using the *Quality Assurance Concern* (QAC) workflow in Qualtrax®.

A complaint is an expression of dissatisfaction by any person or organization to a laboratory, relating to the activities or results of that laboratory, where a response is expected. The ASCL considers complaints to be useful in identifying opportunities for improvement.

7.9.2 TRANSPARENCY OF PROCESS

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding transparency of process.

7.9.3 COMPLAINT PROCESS

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding the complaint process.

7.9.4 RESPONSIBILITY

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding responsibility.

7.9.5 COMMUNICATION

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding communication.

7.9.6 INDEPENDENT EVALUATION

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding independent evaluation.

7.9.7 NOTICE OF COMPLETION

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding notice of completion.

7.10 NONCONFORMING WORK

7.10.1 GENERAL

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding nonconforming work.

There are three key levels of non-conforming work, each of which may require a different response:

- Simple corrections
- Level 2 nonconformities
- Level 1 nonconformities

7.10.1.1 SIMPLE CORRECTION

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding simple corrections.

7.10.1.2 LEVEL 2 NONCONFORMITY

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding Level 2 nonconformities.

7.10.1.3 LEVEL 1 NONCONFORMITY

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding Level 1 nonconformities.

7.10.2 RECORDS OF NONCONFORMING WORK

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding records of nonconforming work. Simple corrections can be documented in the case file or discipline's quality control records, when appropriate.

7.10.3 CORRECTIVE ACTION IMPLEMENTATION

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding corrective action implementation.

7.11 CONTROL OF DATA AND INFORMATION MANAGEMENT

7.11.1 ACCESS TO INFORMATION

The laboratory will have access to the data and information needed to perform laboratory activities.

7.11.2 LIMS VALIDATION

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding LIMS validation.

7.11.2.1 LABORATORY-DEVELOPED SOFTWARE

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding laboratory-developed software.

7.11.3 LIMS REQUIREMENTS

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding LIMS requirements.

7.11.4 OFF-SITE LIMS

The ASCL's LIMS is managed and maintained on the ASCL's main premise.

7.11.5 LIMS DOCUMENTATION

JusticeTrax® contains a help file, and maintains a Customer Care help center which contains updated information.

7.11.6 CALCULATIONS AND DATA TRANSFERS

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding calculations and data transfers.

7.11.6.1 CALCULATION AND DATA TRANSFER RECORDS

See *ASCL Quality Manual* (ASCL-DOC-01) for further information regarding calculation and data transfer records.

8 MANAGEMENT SYSTEM REQUIREMENTS

8.1 OPTIONS

8.1.1 GENERAL

The ASCL has a management system capable of supporting and demonstrating the consistent achievement of all accreditation requirements and assuring the quality of laboratory results.

8.1.2 OPTION A

The ASCL opts for Option A, and addresses the following topics:

- Management system documentation
- Control of management system documents
- Control of records
- Actions to address risks and opportunities
- Improvement
- Corrective actions
- Internal audits
- Management reviews

8.1.3 OPTION B

The ASCL is not accredited to ISO 9001, and does not opt for Option B.

8.2 MANAGEMENT SYSTEM DOCUMENTATION (OPTION A)

8.2.1 POLICIES AND OBJECTIVES

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding policies and objectives.

All personnel are responsible for knowing and using the policies and procedures in their Discipline Quality Manual. Each Discipline Quality Manual and Discipline Training Manual are reviewed annually by the appropriate Section Chief and updated as needed.

8.2.1.1 REQUIREMENT FOR WRITTEN EVIDENCE

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding requirement for written evidence.

8.2.2 MISSION AND QUALITY POLICY STATEMENTS

The mission of the Arkansas State Crime Laboratory is to provide the highest quality scientific services to the criminal justice community and the State of Arkansas. This is accomplished through a team of skilled and dedicated employees using scientific equipment and appropriate validated methodologies. The laboratory strives to provide these services in a timeframe amenable to our customers.

PHYSICAL EVIDENCE

SEROLOGY UNIT MISSION

Utilize scientific methodologies and instrumentation to examine physical evidence for the presence of blood, semen and/or transfer DNA. Collect and store tape lifts for possible further testing.

See next page for ASCL Quality Policy Statement.

QUALITY POLICY STATEMENT

The goal of the Arkansas State Crime Laboratory (ASCL) is to provide the highest quality forensic services to our customers. The ASCL has defined its customer base as the Judicial System, which includes law enforcement agencies, prosecutors and defense counsel, and regulatory and other public service government agencies. The ASCL is committed to meet the needs and expectations of our customers through a dedication to quality and service.

The ASCL standard of quality requires that all forensic conclusions, both written and oral, are scientifically valid, accurate, consistent, and reliable. This standard of quality serves as the guiding principle for all technical and strategic decisions involving work undertaken by the ASCL.

This guiding principle is shared by all employees of the ASCL.

The objectives involved in meeting our quality goal are:

- Ensuring the use of validated procedures that are reliable, reproducible, and which serve their intended purpose with respect to precision, accuracy, sensitivity, and specificity
- Providing laboratory reports that are clear, accurate, objective, and readily understood by our customers
- Providing relevant, professional, and impartial testimony in judicial proceedings
- Participating in a proficiency testing program that monitors the capabilities of the analysts/examiners and the reliability of our analytical results
- Participating in annual audits of the quality system
- Providing a system to ensure the integrity and security of evidence from its receipt to its return
- Complying with ANAB Accreditation Standards
- Continually improving the effectiveness of the ASCL Quality Management System
- Identifying opportunities for improvement related to quality in all areas of operation, taking corrective action to remediate non-conforming work, and striving to prevent recurrence
- Providing continuing employee education and training

The entire staff of the ASCL will adhere to the spirit and intent of the quality assurance program, as well as to the directives of this Quality Manual and its supporting documents, which include the Personnel Handbook, the Health and Safety Manual, and the Discipline Quality and Training Manuals. All members of the staff will strive to improve customer satisfaction for every service provided by this laboratory.

We are committed to a strategy of continuous improvement: constantly determining the needs and expectations of our customers and striving to meet them.

I personally affirm these commitments and support the established comprehensive quality assurance system, which will allow our agency to meet all of the requirements of the ANAB Accreditation Standards.

Kermit B. Channell, II

8.2.3 COMMITMENT TO MANAGEMENT SYSTEM

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding commitment to management system.

8.2.4 DOCUMENTATION

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding documentation.

8.2.5 ACCESSIBILITY

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding accessibility.

8.3 CONTROL OF MANAGEMENT SYSTEM DOCUMENTS (OPTION A)

8.3.1 CONTROLLED DOCUMENTS

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding controlled documents.

8.3.2 CONTROLLED DOCUMENT POLICIES AND PROCEDURES

8.3.2.1 DOCUMENT APPROVAL

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding document approval.

8.3.2.2 DOCUMENT REVIEW

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding document review.

8.3.2.3 DOCUMENT REVISION

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding document revision.

8.3.2.4 DOCUMENT AVAILABILITY

All documents are available in the Qualtrax® document control system. Access to view documents is not controlled. Revision of documents is only possible through the process outlined in section 8.3.2.3.

8.3.2.5 DOCUMENT IDENTIFICATION

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding document identification.

8.3.2.6 DOCUMENT OBSOLESCENCE

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding document obsolescence.

Employees will destroy outdated documents upon receiving updated documents. It is the employee's responsibility to verify that they are using the current revision of any document.

8.4 CONTROL OF RECORDS (OPTION A)

8.4.1 RECORDS

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding records.

TECHNICAL RECORDS

Case files will be retained by the Arkansas State Crime Laboratory in either physical or electronic form. The Arkansas State Crime Laboratory uses the JusticeTrax® LIMS-plus software program. All case documentation will be stored electronically. Once reviewed, this electronic version is considered the official case record.

Historical non-electronic case files for Serology are stored within the section; in the file room in the main building; in the evidence storage area in Evidence Receiving; or in the file rooms located in the annex.

QUALITY RECORDS

Quality records, such as reagent and chemical QC logs, are stored in a Reagent Prep Logbook binder within the Serology Unit, and are accessible to all Physical Evidence Section employees.

Quality records, such as in-house training records, are stored in the *Serology Training Manual* (SER-DOC-02) binders within the Serology Unit, and are accessible to the Section Chief and any designees.

8.4.2 RECORD POLICIES AND PROCEDURES

RECORD RETENTION

Case files will be stored indefinitely. The following items are required to be retained (either electronically or physically) for a period of eight years:

- Quality Assurance Concern Documentation
- Audit Records
- Training Records
- Continuing Education Documentation
- Proficiency Testing Records
- Court Testimony Reviews

All other quality records (e.g., Qualtrax®, instrument maintenance logs) will be stored for at least one full ASCLD/LAB-*International* accreditation cycle (four years).

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding record retention.

8.5 ACTIONS TO ADDRESS RISKS AND OPPORTUNITIES (OPTION A)

8.5.1 RISKS AND OPPORTUNITIES

The ASCL considers risks and opportunities in several ways:

- Preventive actions identified by the *Quality Assurance Concern* (QAC) workflow
- Evaluation of risks and opportunities during periodic state-mandated Risk Assessment activities
- Internal audits
- Identification of risks and opportunities during management review
- External comments and complaints
- Internal comments and complaints
- Customer surveys

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding risks and opportunities.

8.5.1.1 HEALTH AND SAFETY

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding risks and opportunities related to health and safety.

8.5.2 PLANNING

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding planning.

8.5.3 PROPORTIONALITY

The actions taken to address risks and opportunities will be proportional to their potential impact on the validity of laboratory results.

8.6 IMPROVEMENT (OPTION A)

8.6.1 IMPROVEMENT

The laboratory shall strive to continually improve the effectiveness of the Quality Management System. Opportunities for improvement are identified through various means, including:

- *Quality Assurance Concern* submissions

- Corrective and Preventive Action Requests
- Customer surveys
- Annual management reviews
- Annual document review
- Internal and external audits
- Employee suggestions

8.6.2 EXTERNAL FEEDBACK

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding external feedback.

8.7 CORRECTIVE ACTIONS (OPTION A)

8.7.1 NONCONFORMITIES

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding nonconformities.

8.7.2 PROPORTIONALITY

Corrective action will be appropriate to the type and severity of the nonconformity and/or its effects. A serious nonconformity may necessitate an additional audit of the appropriate area(s), or of the entire quality system, if it brings compliance with established policies and procedures or ANAB accreditation requirements into question. These additional audits may be from an external source or conducted internally.

8.7.3 RECORDS

The corrective action process is documented and maintained using the Qualtrax® system. This record shall include a description of the nonconforming work, the effect of the discrepancy, root cause findings, the action(s) taken, the results of corrective action, and any after-action monitoring requirements to avoid recurrence.

8.8 INTERNAL AUDITS (OPTION A)

8.8.1 INTERNAL AUDITS

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding internal audits. Internal audits of the laboratory will be performed to verify that laboratory operations comply with the requirements of the management system and accreditation requirements.

8.8.1.1 SCHEDULE

Internal audits shall be conducted each calendar year. Horizontal items may occur throughout the year.

8.8.2 AUDIT POLICIES AND PROCEDURES

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding audit policies and procedures.

8.9 MANAGEMENT REVIEWS (OPTION A)

8.9.1 MANAGEMENT REVIEW

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding management review.

8.9.1.1 TIME FRAME

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding timeframe.

8.9.2 INPUTS

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding inputs.

8.9.3 OUTPUTS

See *ASCL Quality Manual* (ASCL-DOC-01) for general information regarding outputs.

9 SEROLOGY UNIT TEST METHODS

9.1 GENERAL

In accordance with the ASCL Mission Statement, it is the goal of the Serology Unit to process evidence in agreement with the *ASCL Case Management Guidelines* when possible. It is understood that some cases will require more testing, based on specific case information and/or the nature of the crime. However, examination of the most probative and non-redundant evidence items should be prioritized.

In general, for case types other than Sexual Assaults, the top five most probative samples will be examined. For Sexual Assault cases, a Sexual Assault Evidence Collection Kit will generally be examined before any other items. Refer to the *ASCL Case Management Guidelines* (ASCL-DOC-10) for details.

The Serology Unit uses appropriate methods for all testing and evidence handling that meet the needs of the customer. The following methods encompass the most commonly encountered evidence types and are intended to serve as guidelines to analysts. Analysts have discretion in choosing appropriate procedures for a particular piece of evidence.

CONTAMINATION PREVENTION PROCEDURES

- Approved Cleaning Supplies: Bleach-Rite ® (contains at least a 1:10 dilution of NaOCl, bleach; 0.55% by weight), Clorox ® Germicidal Wipes (0.55% NaOCl), or a 10% solution of bleach may be used to clean laboratory surfaces for contamination prevention[54].
- Examination areas and tools, such as forceps and scissors, shall be in clean condition prior to use during evidence examination, at minimum, but may be cleaned more frequently as necessitated by the immediate case under examination.
- Clean swabs, test tubes, disposable pipettes, slides, etc. are stored in the working areas such that no contact between those items and evidence will occur.
- Any person coming within close proximity of a serologist's bench top while the serologist has evidence on the bench top shall wear necessary personal protective equipment (PPE) to prevent contamination.
- Prior to, during, and/or after evidence preservation and testing processes, change gloves and wipe off examination/work areas (i.e., countertops, drawer handles, cabinets), tools (i.e., tweezers, scissors, pipettes), and tube racks with approved cleaning supplies (see approved cleaning supplies bullet above).
- Serologists shall wear disposable gloves, a laboratory coat, and a facemask during the examination of evidence and collection of biological stains, hairs, and fibers.

[54] To prevent corrosion of metal surfaces and deter sodium hypochlorite crystal build-up, it is recommended that analysts rinse surfaces and items with distilled water after being bleached with either approved cleaning supply.

- Gloves shall be changed, when appropriate, to prevent unintentional transfer of biological materials from one evidence item to the next.
- Laboratory coats shall be changed, when appropriate, to prevent unintentional transfer of biological materials from one evidence item to the next. (This is generally determined by location where the evidence was collected, (i.e., scene location) or known ownership of the evidence, (i.e., victim or suspect)).

- Serologists shall remove disposable gloves prior to handling or using those areas designated as "CLEAN" in the laboratory. Labels designating certain items as clean have been placed on most areas, door handles, and items that are considered clean, meaning that gloves shall not be worn when handling these items. Alternately, "BIOHAZARD" labels have been placed on those items that are not clean meaning that gloves shall be worn when handling items or using areas labeled as biohazard.

Clean items include, but are not limited to, the following:

- FD Secure Storage Cabinet
- FD Cold Storage refrigerator
- PE Secure Storage Cabinet
- ALL supply cabinets and drawers
- Copier and Printers
- Notebooks/Logbooks

Biohazardous items include, but are not limited to, the following:

- Water baths
- Ovens
- Slide warmers
- ALS devices
- Refrigerators
- Centrifuges
- Glass waste containers
- Biohazard container
- Sharps container

9.1.1 SEROLOGY UNIT SAMPLING METHOD

Any sampling of items of evidence that occurs within the test methods used by the Serology Unit of the Arkansas State Crime Laboratory is nonstatistical sampling[55]. With this type of sampling, there is no assumption of homogeneity of the whole. Sampling only answers questions about the portion tested. The analyst's approach is based on training and experience. (See each applicable section 9 Test Method's "Sample Preparation" section for specific sampling information.)

[55] This was formerly referred to as sample selection.

GENERAL GUIDELINES: SCREENING FOR BIOLOGICAL STAINS

- Review the summary provided on the submission sheet. Review officer's report (if submitted) and speak with the detective or attorney if necessary.
- Determine whether the evidence should be examined first by the Trace Evidence Unit. Communicate the needs of the case with an analyst in the Trace Evidence Unit so that materials such as gunshot residue primer, glass, paint, hairs, or other trace materials may be collected and preserved before biological examinations begin.
- Determine whether the evidence submitted for potential biological materials has additional requests for analysis by the Latent Prints Section and/or the Firearms & Tool Marks section, as this may affect the method in which the evidence will be handled and/or marked in the Forensic Serology Unit.
- Cover work surface with clean exam paper prior to examining evidence.
- Clean scissors and tweezers/forceps with 10% bleach then rinse with water.
- Document and label all packaging.
- Open package without destroying other seals and initials when possible.
- Describe item in case notes.
- Diagram or photograph item if helpful in creating a record of evidence. An infrared camera may be used to photograph evidence. Infrared photography is best suited for photographing stains on dark colored fabric or clothing. Ideal light sources for infrared photography are: sunlight through a window, the camera flash, and incandescent light. Photography under fluorescent light alone is not suggested, because there is a low amount of infrared light emitted. After a photograph is taken, its appearance may be enhanced by converting the color scheme to grayscale on a computer. If the fabric type is suitable for infrared photography, the fabric should appear to be light gray while the stains remain dark gray.
- Visually examine the item for possible biological material. An alternate light source (ALS) may be used when no potential seminal stains are observed with the unaided eye. Chemically test (e.g., phenolphthalein and/or AP) any stains of interest. Document results in case notes.
- Evaluate each possible biological stain to determine the appropriate amount to be consumed in testing. Conserving material for future testing is a priority. If the stain is small enough in size that consumption of the entire stain by testing is a concern, the analyst may choose to retain the stain after conducting presumptive testing only or no testing at all. Notes should adequately reflect reasons for this limited testing approach.
- When appropriate, the approximate sizes of bloodstains and semen stains should be documented in the case notes[56]. Bloodstains can be described according to size, directionality, surface of origin (inside or outside a garment), etc.

- ***Note***: Any literature references cited in section 9 Serology Unit Test Methods can be found either on the Arkansas State Crime Laboratory website, other listed webpages, or in the Serology Training Program Literature Binder which is maintained within the Serology Unit. In

[56] Photographs may be used for this documentation.

addition, some handbooks and textbooks are also stored electronically on Qualtrax® and physical copies may be found within the Physical Evidence Section Units.

9.2 COLLECTION OF HAIRS AND FIBERS

9.2.1 SCOPE

Hairs and fibers should be collected when deemed necessary by the analyst after consideration of information presented in the case. The investigating officer and/or the Trace Evidence analyst may be consulted. This is a collection phase only—microscopic analysis may be conducted by a qualified analyst at another juncture[57]. If tape lifts are taken, they may be used for future hair analysis, fiber analysis, and/or DNA (transfer or touch DNA) analysis.

9.2.2 REAGENTS, CHEMICALS, STANDARDS, AND CONTROLS

As no testing occurs during the collection of hairs and fibers, there are no reagents or chemicals involved in the method.

FABRIC STANDARDS/DNA SUBSTRATE CONTROLS

Known fabric standard samples including all the fiber types and colors (when feasible) are cut from fabric items and are either placed on a transparency sheet with clear tape or placed in a coin envelope. White cotton, denim, light-colored fabrics, and smooth fabrics (such as nylon windbreakers) are not suitable target fibers.

> ***Note***: As fabric standard cuttings may also serve as a DNA substrate control area for the Forensic DNA section's use when troubleshooting, whenever possible select unstained areas for cutting.

If no tape lifts are retained from an item of evidence, but biological samples are retained (e.g., touch/transfer DNA swabs, stain cuttings or swabs), a control cutting shall be retained from porous/absorbent substrates (fabrics, untreated paper, etc.) for possible use by the Forensic DNA Section. Due to their construction, it is not mandatory to retain control cuttings from footwear, but if the upper portion of the footwear appears porous/absorbent and can be cut, it is *best practice* to retain a control cutting.

SUBSTRATE CONTROLS

A substrate control is collected from a non-stained area in close proximity, when possible, to the stain(s) being retained. This sample is used to ensure that the substrate itself does not interfere

[57] Hair identification, including DNA suitability, is conducted by the Trace Evidence Unit at the ASCL when requested by the Serology Unit or the DNA Section. All other types of hair/fiber analysis may be conducted by an outside laboratory if requested by the customer. Serologists should always preserve probative hair/fiber evidence for possible future analysis.

with laboratory tests. Substrate controls are collected when tape lifts, cuttings, or swabs are retained from an item.

Substrate controls are not necessary under certain circumstances:

- No swabs, cuttings, or tape lifts are retained from an item.
- The questioned item is a hard surface.
- In the case of tape lifts (with no touch DNA potential), substrate controls are not necessary for denim, 100% white cotton fabric, light colored fabrics, or smooth fabrics such as nylon windbreakers; however, if a cutting or swab of the fabric is retained (or tape lifts will be submitted) for DNA testing, a substrate control must be collected.

1) Use clean scissors or a sterile surgical blade.
2) Cut a sample, approximately 1 inch by 1 inch (if possible), from an unstained area on the item.
3) If the control cutting will be retained with the DNA cuttings, place the cutting into a clean envelope (coin envelope, #1) and label the exterior front of the envelope with the following information:

- Arkansas State Crime Laboratory Case Number
- Evidence Number
- Description of Control (optional)
- Analyst Initials
- Initial seal on envelope

4) If the control cutting will be retained with the tape lifts, place the cutting onto clear packaging tape and adhere to the acetate sheet containing tape lifts from the item. Label appropriately.
    - Retained controls shall be documented in analyst's notes.

9.2.3 SAMPLE PREPARATION

9.2.3.1 EVIDENCE ASSESSMENT BY ANALYST

1) Determine which items are from the victim, suspect, scene, etc. Review the "type of analysis requested" section of the *Evidence Submission Form* (ASCL-FORM-12).
2) Primer GSR -If **suspect clothing** was submitted and a firearm was discharged during the incident, **DO NOT** tape lift any items until contact with the Trace Evidence Unit and/or investigating agency has established whether primer GSR sampling should be conducted. Serological handling of the items can detrimentally affect the outcome of GSR collection/testing. In the cases where primer GSR should be collected, transfer the appropriate evidence to a Trace Evidence analyst before proceeding with any collection or testing in Serology.
3) Distance Determination- If **victim clothing** was submitted and the victim was wounded by gunfire and a firearm was submitted, **DO NOT** tape lift any items until contact with the

Firearms and Tool Marks Section and/or investigating agency has established whether distance determination should be conducted. Serological handling of the items can detrimentally affect the outcome of distance determination. It is usually acceptable to tape lift in areas away from the possible bullet holes. However, consult with a Firearms and Tool Marks examiner before proceeding.

4) Whenever possible, hair and fiber collection should be conducted upon the initial opening of evidence and before any other types of testing are conducted. This approach gives analysts the best opportunity to preserve hair/fiber evidence that could be lost due to positioning and/or swabbing of evidence.
5) Hairs and fibers should generally not be collected on items where the victim(s) and suspect(s) are known to have been living together. In this circumstance, it may, however, be necessary to examine some items (i.e., murder weapon) for a transfer of hairs and/or fibers.

9.2.3.2 METHOD

COLLECTION FROM SEXUAL ASSAULT KITS

1) Examine any kit swabs that are to be collected for DNA analysis for hair/fiber evidence. If located, place any hair/fiber evidence in a Kimwipe or folded piece of paper and tape seal in a labeled coin envelope. Document retained hair/fiber evidence in notes.
2) Examine the contents of the "Underwear" bag. Tape lift contents on labeled transparency sheets. Hair/fiber evidence may also be directly removed from underwear and stored in a Kimwipe or folded piece of paper and tape seal in a labeled coin envelope. Return "Underwear" bag to the kit. Document retained tape lifts and/or directly removed hair/fiber evidence in notes.
3) Known fabric standard samples including all the fiber types and colors (when feasible) are cut from the item and are either placed on a transparency sheet with clear tape or placed in an envelope. White cotton, denim, light-colored fabrics, and smooth fabrics (such as nylon windbreakers) are not suitable target fibers.
   Note: As fabric standard cuttings may also serve as a DNA substrate control area for the Forensic DNA section's use, when possible select unstained areas for cutting.
4) The Pubic Hair Combings will not routinely be examined but will remain in the kit. It will be documented as "also submitted, not examined (ASNE)" in the analyst's notes. An exception may be made based on communication from the investigating agency. In these cases the following procedures are followed: Examine the contents of the "Pubic Hair Combings" envelope. Remove all hairs from the comb and/or napkin. Place the hairs in a Kimwipe or folded piece of paper and tape seal in a labeled coin envelope. Return the "Pubic Hair Combings" envelope to the kit. Document retained hair/fiber evidence in notes.
5) Place tape lift transparency sheets and the pubic hair combings coin envelope (if applicable) in a large, tape sealed manila envelope and label accordingly.

6) Note any nonstandard kit envelopes that may have been added by medical personnel for specific hair/fiber collection. Generally, these items will not be examined but will remain in the kit. They will be documented as "also submitted, not examined (ASNE)" in the analyst's notes. An exception may be made based on communication from the investigating agency. In these cases, retain any additional hair/fiber evidence envelopes (unopened and unexamined) in the same manila envelope as the underwear tape lifts and pubic hair combings. The evidence # of the kit and the body site/contents description written on the respective envelopes may be used for the identity of these items[58]. Document retained unopened/unexamined envelopes in notes.
   Note: If kit samples are marked as being collected according to the information provided on the envelopes/bag, but the contents are empty, document the item's empty contents status in notes. (e.g., Q4 Pubic Hair Combings-no hairs present)
7) Return all opened kit envelopes/bags to the kit in a labeled, sealed, and initialed condition. It is also best practice to label envelopes that were not examined with the case # and analyst's initials, at a minimum.
8) Review evidence collected. If known hair/fiber samples have been submitted and/or additional work needs to be completed, contact the appropriate analysis section or turn the case over to the section supervisor for reassignment.

COLLECTION FROM CLOTHING OR OTHER ITEMS

1) Visually examine item and note description of item and fabric content, if applicable.
2) Be cautious in preserving evidence that other sections may need to examine (i.e., blood stains or latent prints). It may be necessary to collect fibers and/or hairs with forceps and place in an envelope or on tape rather than tape lifting the item directly.
3) Tape lifting is accomplished by taking a section of clear adhesive tape and pressing on the item and pulling away. Fibers and/or hairs adhere to the tape, which is then placed on a clear transparency sheet. Continue collecting with sections of tape until the entire item has been covered.
4) Label the transparency sheet with ASCL case #. Near each beginning set of tape lifts, label with Q#-TL and the name of the item that was tape lifted. If intentionally segregated surfaces/regions of an item are tape lifted, label this location information (i.e., inner, outer, outer left sleeve) as it is helpful information for the Forensic DNA section to have when tape lifts are swabbed for touch/transfer DNA.
5) Known fabric standard samples including all the fiber types and colors (when feasible) are cut from the item and are either placed on a transparency sheet with clear tape or placed in an envelope. White cotton, denim, light-colored fabrics, and smooth fabrics (such as nylon windbreakers) are not suitable target fibers.

[58] e.g., E-4 Hair from Sock Cuff

Note: As fabric standard cuttings may also serve as a control area for the Forensic DNA section's use, when possible select unstained areas for cutting.

6) Place envelopes and/or tape lift transparency sheets in a large manila envelope. Label the envelope with the ASCL case number, evidence number(s), analyst's initials, agency name, agency case number, and offense type. Any other labeling is considered optional.
7) Review evidence collected. If known hair/fiber samples have been submitted and/or additional work needs to be completed, contact the appropriate analysis section or turn the case over to the section supervisor for reassignment.

9.2.4 QUALITY ASSURANCE/CONTROL MEASURES

As with all Serology Unit methodology, using clean techniques when approaching the collection of hairs and fibers is imperative to the procedure. The Serology Training Program covers all aspects of preventing contamination as well as the proper techniques to both evaluate evidence submissions and collect tape lift and hair/fiber samples from evidence. Additional hair and fiber training is also conducted by the Trace Evidence Unit for new analysts hired in the Serology Unit. Feedback is also given by the Forensic DNA Section should occurrences of contamination be identified, which allows for reevaluation and improvement of the analyst's techniques.

9.2.5 INTERPRETATION OF RESULTS

9.2.5.1 PRECAUTIONS

As this is a collection phase of evidence only, there are no results to interpret. However, this section describes some general precautions to consider. It is the analyst's responsibility to ensure that hair/fiber evidence is neither lost nor unintentionally added while evidence items are being examined.

- Be aware of the potential for static. Hairs/fibers may become statically charged and therefore attracted to gloves, forceps, or other items within close proximity. For this reason, the analyst should exercise caution when handling this type of evidence.
- Prior to opening evidence, observe the surfaces of the laboratory coat to be worn to ensure no stray hairs or fibers are present that could be inadvertently transferred to the evidence.
- Analysts should take appropriate measures to ensure that their own hair does not contaminate the evidence.
- Analysts should refrain from leaning over their examination surfaces whenever practicable.

9.2.5.2 POSSIBLE SOURCES OF ERROR

As this is a collection phase of evidence only which does not involve interpretation of results, there are no applicable possible sources of error to consider.

9.2.5.3 LITERATURE REFERENCES

- Physical Evidence – Trace Unit Quality Manual (TR-DOC-01) section 9.4 Hair and Fiber Collection
- Saferstein, *Criminalistics*, 6th Ed., Case Study – The "Bobby Joe" Long Serial Murder Case, pp 550-565
- Physical Evidence – Trace Unit Training Manual (TR-DOC-02 section 4.5.3 Reading Assignments):
  - Scientific Working Group on Materials Analysis (SWGMAT), Evidence Committee, "Trace Evidence Recovery Guidelines, January 1998 revision", *Forensic Science Communications*, October 1999, Vol. 1, No. 3, p. 1–10.
  - Saferstein, R., *Forensic Science Handbook, Vol. II*, Prentice Hall, 1998, pp. 164-168, 218-221.
  - Lowrie, C. N., and Jackson, G., "Recovery of Transferred Fibres," *Forensic Science International*, 1991, Vol. 50, pp. 111-119.
  - Lowrie, C. N., and Jackson, G., "Secondary Transfer of Fibres," *Forensic Science International*, 1994, Vol. 64, pp. 73-82.
  - Pearson, E.F., et al, "Glass and Paint Fragments Found in Men's Outer Clothing—Report of a Survey," *Journal of Forensic Sciences*, Vol. 16, No. 3, July 1971, pp. 283-300.
- *Forensic Biology*, 2nd Ed. Chapter 1, 4
- ASCL-DOC-10 *Case Management Guidelines*

9.2.5.4 CRITERIA FOR RESULTS

As this is a collection phase only, there are no applicable criteria for interpretation of results.

9.2.6 NOTES/DOCUMENTATION REQUIREMENTS

1) Adequately describe packaging in notes.
2) Adequately describe evidence items in notes[59]; include fabric content if applicable.
3) Photographs or photocopies of the items and/or packaging may be taken, but isn't mandatory.
4) Document any retained samples (e.g., hairs, fibers, tape lifts, debris) in notes.

9.2.7 REPORT WRITING

The current Serology Autotext document is maintained on the S: drive and is used as a model for the Serology report writing process in JusticeTrax® LIMS. It contains all of the common wording options for Serology reports. Wording is selected by the analyst depending on the case/analysis type and communicates what was tested, the results of those tests, what was not examined, any DNA reference samples that may be needed, all retained items, and informs the customer of future

[59] Items should be described with enough detail so that an analyst testifying as a substitute has adequate information to give appropriate testimony to the jury.

DNA analysis, if applicable. This wording is intended to meet the needs of the vast majority of cases encountered. However, wording may be tailored as needed when non-routine or unique information also needs to be reported. The Physical Evidence Section Chief may be consulted to aid in determining this wording, if needed.

RESULTS CHART

The evidence item that has tape lifts and/or hair or fiber evidence collected from it and any retained (unopened/unexamined) hair/fiber envelopes will be listed in the Serology Chart under the "Results" section of the report.

RETAINED TAPE LIFTS

Under the "Retained Samples" section of the report, the appropriate Q#(s) will be listed on the "Tape lift(s) from…" line.

RETAINED HAIRS, FIBERS, AND/OR DEBRIS

Under the "Retained Samples" section of the report, the appropriate Q#(s) will be listed on the "Hair(s) from…" line. If a combination of hairs and fibers are retained, the line may be edited to read "Hair(s)/Fiber(s) from…" If the items retained are generally unidentifiable, "Debris from…" is appropriate for use.

Note: Hairs that are directly removed by hand from an item should be reflected in the "Hair(s) from…" line. However, hairs removed from tape lifts are represented by the "Tape lift(s) from…" line and should not be separately referenced on the "Hair(s) from…" line.

RETAINED (UNOPENED/UNEXAMINED) HAIR/FIBER ENVELOPES

Under the "Retained Samples" section of the report, the appropriate E#(s) will be listed on the "Item(s)…" line.

9.2.8 CRITICAL REAGENTS AND EQUIPMENT SPECIFICATIONS

There are no critical reagents or equipment associated with the collection of hairs and/or fibers.

9.3 COLLECTION OF STAINS FOR FURTHER TESTING

9.3.1 SCOPE

Stains should be collected when deemed necessary by the analyst after consideration of information presented in the case. The investigating officer may be consulted. This is a collection phase only.

9.3.2 REAGENTS, CHEMICALS, STANDARDS, & CONTROLS

As no testing occurs during the collection phase of stains, there are no reagents or chemicals involved in the method.

FABRIC STANDARDS/DNA SUBSTRATE CONTROLS

Known fabric standard samples/DNA substrate control cuttings are retained from porous items from which biological stains are also being retained. Whenever possible select unstained areas for cutting[60]. (See sections 9.2.2 and 9.2.3.2 (c)).

9.3.3 SAMPLE PREPARATION

9.3.3.1 EVIDENCE ASSESSMENT

Determine which items are from the victim, suspect, scene, etc. Review the "type of analysis requested" section of the evidence submission form. There are three methods of collecting stains, which may contain DNA, from a substrate: cutting, swabbing, and tape lifting.

9.3.3.2 METHOD

COLLECTION BY CUTTING

Cutting is useful for porous substances such as clothing, paper, upholstery, etc.

Note: Cuttings from clothing may assist in developing a DNA profile from the owner of the clothing instead of the most recent wearer of the clothing.

1) Use clean tools (e.g., scissors, scalpel blades, etc.) according to Section 5.4.
2) Cut the stain from the whole substrate.
    - Small stains: Cut the entire stain.
    - Large stains: Cut a portion(s) of the whole stain taking a representative sample.
    - Semen stains: Cut semen stains where the highest concentration of spermatozoa may be located; use alternate light source to aid in stain collection if necessary.
3) Place cutting into a clean envelope (coin envelope, #1). Small cuttings may first be placed into a paper-fold and then into the clean envelope. Other appropriate packaging may also be used.
4) Label the exterior front of the coin envelope with the following information:
    - Arkansas State Crime Laboratory Case Number
    - Evidence Number
    - Description of Retained Evidence (optional)
    - Analyst Initials

[60] On occasions, an item may have no unstained areas from which to retain a suitable substrate control cutting. Analyst notes should reflect this information (i.e., "No suitable area for control cutting.")

5) Initial the tape seal on envelope

COLLECTION BY SWABBING

Swabbing is useful for non-porous substrates such as cans, plastics, glass, vinyl, etc.

Note: Swabbing clothing (inner surfaces and/or skin contact points) may assist in developing a DNA profile from the most recent wearer of the clothing instead of the owner of the clothing.

1) Moisten a sterile swab with deionized water.
2) Rub the stain with the swab until the stain is completely collected or the swab is saturated thoroughly with the stain. Repeat steps 1 and 2 until enough of the stain has been collected.
3) Allow swab(s) to air dry.
4) Place swab(s) into a clean envelope (coin envelope, #1). Other appropriate packaging may also be used.
5) Label the exterior front of the coin envelope with the following information:
    - Arkansas State Crime Laboratory Case Number
    - Evidence Number
    - Description of Retained Evidence (optional)
    - Analyst Initials
6) Initial the tape seal on envelope

COLLECTION BY TAPE LIFTS

This technique may be used to collect epithelial (skin) cells from porous surfaces such as clothing, bedding, upholstery, etc.

1) Obtain a section of clear packaging tape approximately 3–4 inches in length and fold over a portion of tape to assist in removal.
2) Apply the tape to the surface to be sampled using slight pressure and then pull away. Continue to apply tape and pull away from substrate until the area to be sampled has been covered.
3) Place tape section onto clear acetate and label with the following information:
    - Arkansas State Crime Laboratory Case Number
    - Evidence Number
    - Description of Evidence (optional)
4) Place tape lifts into a clean envelope.
5) Label the exterior front of the envelope with the following information:
    - Arkansas State Crime Laboratory Case Number
    - Evidence Number(s)
    - Analyst Initials
    - Agency & case number
    - Offense
6) Initial seal on envelope
7) Review evidence collected.

8) If additional work needs to be completed, submit to the appropriate analysis section.

9.3.4 QUALITY ASSURANCE/CONTROL MEASURES

As with all Serology Unit methodology, using clean techniques when approaching the collection of stains is imperative to the procedure. The Serology Training Program covers all aspects of preventing contamination as well as the proper techniques to both evaluate evidence submissions and collect stains from evidence. Feedback is also given by the Forensic DNA Section should occurrences of contamination be identified, which allows for reevaluation and improvement of the analyst's collection techniques.

9.3.5 INTERPRETATION OF RESULTS

9.3.5.1 PRECAUTIONS

As this is a collection phase of evidence only, there are no results to interpret. However, this section describes some general precautions to consider. It is the analyst's responsibility to ensure that stain evidence is neither lost nor unintentionally added while evidence items are being examined.

- Clean gloves and lab coat shall be worn and changed when appropriate, based on the analyst's training and experience, to prevent unintentional cross-contamination between items of evidence and laboratory surfaces/equipment.
- Evidence from suspects and victims shall be examined at separate times and/or on different surfaces to prevent unintentional cross-contamination between items of evidence. As this is part of the serological training program, documentation of these precautions is not mandatory in the analyst's notes.

9.3.5.2 POSSIBLE SOURCES OF ERROR

As this is a collection phase of evidence only which does not involve interpretation of results, there are no applicable possible sources of error to consider. However, particular attention should be paid to what is requested by the customer as well as any scenario information which may aid the analyst in determining what types of collection may be useful in the investigation. Clean techniques are required for proper collection.

9.3.5.3 LITERATURE REFERENCES

ASCL-DOC-10 *Case Management Guidelines*: Physical Evidence-Serology and Touch DNA Policy

9.3.5.4 CRITERIA FOR RESULTS

As this is a collection phase only, there are no applicable criteria for interpretation of results.

9.3.6 NOTES/DOCUMENTATION REQUIREMENTS

1) Describe packaging and evidence in notes.
2) Photographs, drawings, or photocopies of the items and packaging may be taken.
3) Describe evidence items in notes; include size and location of stain, when applicable.
4) Describe how stain was collected in notes (i.e., retained cutting, retained swab, retained tape lifts).

9.3.7 REPORT WRITING

See section 9.2.7 for general report writing information regarding Serology Autotext.

RESULTS CHART

The evidence item that has stains collected from it (via cuttings, swabbing, and/or tape lifts) will be listed in the Serology Chart under the "Results" section of the report.

RETAINED SAMPLES

Under the Retained Samples section of the report, the analyst will use the appropriate retained sample listing(s) below:

- **Retained Items**: The appropriate Q#(s) will be listed on the "Item(s)…" line.
- **Retained Cuttings**: The appropriate Q#(s) will be listed on the "Cutting(s) from…" line.
- **Retained Swabs**: The appropriate Q#(s) will be listed on the "Swab(s) from…" line.
  Note: Serological testing will not be routinely performed on Property Crime evidence. Notes and appropriate documentation will be maintained in the LIMS; however, a report will typically not be generated with these case types. See section 9.14.7 for more information on processing Property Crime Evidence.

9.3.8 CRITICAL REAGENTS AND EQUIPMENT SPECIFICATIONS

There are no critical reagents or equipment associated with the collection of stains.

9.4 VISUAL EXAMINATION FOR STAINS

9.4.1 SCOPE

A visual examination of evidence is used to identify stains that are characteristic of blood and/or semen. This critical first step leads the analyst to the appropriate further testing of any biological areas of interest on the evidence being examined.

9.4.2 REAGENTS, CHEMICALS, STANDARDS, & CONTROLS

Visual examination of evidence is an assessment activity only and does not involve the use of any reagents, chemicals, standards, or controls.

9.4.3 SAMPLE PREPARATION

9.4.3.1 EVIDENCE ASSESSMENT

Determine which items are from the victim, suspect, scene, etc. Review the "type of analysis requested" section of the evidence submission form to determine which types of stains may be encountered.

9.4.3.2 SAMPLING METHOD

When using the Visual Examination for Stains method, the stains marked are selected based on nonstatistical sampling.

- Nonstatistical Sampling- stains of interest for further body fluid testing are marked during the visual examination of evidence items. This process is based on the analyst's training and experience. This sampling method only answers questions about the portion tested. There is no assumption of homogeneity of all stains on the evidence item[61].

9.4.3.3 SAMPLING RECORDS

As nonstatistical sampling is the only approach taken in sampling of evidence in the Serology Unit, the record of the sampling method is listed in this section. Case notes provide the other relevant information required:

- Date of sampling (case notes date(s))
- Identification of the person performing the sampling (analyst's name or supervised trainee, if applicable)
- Identification and description of sample (Item name, stain #)
- Identification of the sampling location (e.g., diagrams, photographs or stain location descriptions in notes)
- Any deviation, addition, or exclusion from the sampling method and plan[62].

[61] Example: Shirt with four stains. One stain is chosen to be tested based on the analyst's training and experience. The results of the sampled stain do not apply to the other untested stains.
[62] Deviations from the sampling plan and procedures may be requested by the customer or deemed appropriate by the analyst. Any deviations shall be approved in writing by the appropriate Section Chief and maintained in the case record.

9.4.3.4 METHOD

Visually examine the item of evidence for stains characteristic of biological material. In addition to locating stains visually with the unaided eye, an alternate light source (ALS) may also be used.

If a visual examination reveals stains characteristic of biological material, then proceed to the appropriate presumptive and confirmatory testing procedures for the located stains.

VISUAL EXAMINATION FOR BLOOD

If stains are observed which may be blood, the analyst will proceed to chemical testing procedures.

VISUAL EXAMINATION FOR SEMEN

If stains are observed which may be semen, the analyst will proceed to microscopy and/or chemical testing procedures.

See section 9.5 for more information on (ALS) Examination.

EXCEPTIONS

- Condoms will not be tested for the presence of semen. Rather, swabs will be taken separately from both surfaces. See section 9.14.3 for detailed procedures.
- Items received inside sexual assault kits will not be routinely tested for the presence of blood or semen. See section 9.13.1 for detailed procedures.

9.4.4 QUALITY ASSURANCE/CONTROL MEASURES

As with all Serology Unit methodology, using clean techniques when approaching the visual examination for stains is imperative to the procedure. The Serology Training Program covers all aspects of the proper techniques to both evaluate evidence submissions and visually examine evidence for stains (both with and without ALS use) that have the potential for being identified as blood or semen. On-the-job training and experience are the most important factors in developing and refining effective visual examination skills.

9.4.5 INTERPRETATION OF RESULTS

9.4.5.1 PRECAUTIONS

- Stains may be located in non-apparent areas, such as inner pockets under fabric tags, or in rolled cuffs. The analyst is responsible for conducting a thorough examination of the evidence.
- Stains may be mixtures of body fluids, such as blood and semen. Not all characteristics may be seen when such mixtures occur.
- Mixtures of body fluids with non-probative substances (e.g., dirt) may alter the appearance of stains and warrant further testing.

9.4.5.2 POSSIBLE SOURCES OF ERROR

If all surfaces are not viewed, stains may be missed. Analysts should inspect items methodically and carefully, as they were trained, and include their searching in obscured areas (i.e., pockets, under collars).

Some substrate colors may obscure stains. Analysts must consider all influencing factors which may make stain location more challenging and approach the item using the appropriate visual tools that are at their disposal. When visibility is limited, general swabbing may be helpful.

9.4.5.3 LITERATURE REFERENCES

- NFSTC – Testing of Body Fluids (Semen)
- *Forensic Biology*, 2nd Ed. Chapter 14
- NFSTC – Testing of Body Fluids (Blood)
- *Forensic Biology*, 2nd Ed. Chapter 2, 12, & 13

9.4.5.4 CRITERIA FOR RESULTS

As this is a visual examination and does not include testing at this step, there are no criteria for the interpretation of results.

9.4.6 NOTES/DOCUMENTATION REQUIREMENTS

1) *Visual Examination for Blood*: If no stains are located by visual examination of the item, the analyst shall document notes in a way that indicates no stains were located by visual examination that warranted further testing for the presence of blood (i.e., Nothing of Value Noted (NOVN) Blood).
2) *Visual Examination for Semen*: If no stains are located by visual examination of the item, then the item should be examined using an ALS. If no stains are located by either visual examination or by visual examination with an ALS, the analyst shall document notes in a way that indicates no stains were located by visual examination that warranted further testing for the presence of semen (i.e., NOVN Semen).

9.4.7 REPORT WRITING

See section 9.2.7 for general report writing information regarding Serology Autotext.

At the end of the visual examination process, the analyst will determine whether further testing is warranted for body fluid identification. If no further testing is required (i.e., no stains are located that require presumptive and/or confirmatory testing) a report may be created.

RESULTS CHART

The evidence item that was visually examined will be listed in the Serology Chart under the "Results" section of the report.

VISUAL EXAMINATION FOR BLOOD

Using JusticeTrax®, for each "NOVN Blood" item that was examined, the analyst will select "No Visual Stains" from the blood findings drop-down menu. This will populate the blood column on the report to read "No Visual Stains."

VISUAL EXAMINATION FOR SEMEN

Using JusticeTrax®, for each "NOVN Semen" item that was examined, the analyst will select "No Visual/ALS Stains" from the semen findings drop-down menu. This will populate the semen column on the report to read, "No Visual/ALS Stains."

Note: If an item is not tested for a particular body fluid, this may be reported by selecting "Not Tested" or "N/A" in the blood and/or semen findings drop-down menu(s).

If any stains require further serological testing, the report writing step will not occur until all of the testing has been completed.

9.4.8 CRITICAL REAGENTS AND EQUIPMENT SPECIFICATIONS

There are no critical reagents or equipment associated with the visual examination of stains.

9.5 ALTERNATE LIGHT SOURCE (ALS) EXAMINATION

9.5.1 SCOPE

The alternate light source (e.g., Rofin PL400, Rofin Polilight-Flare Plus 2) is a visual tool used to collect trace evidence and make possible semen stains, saliva stains, urine stains, and other body fluids on physical evidence visible. It should not be considered an alternative to chemical tests. Certain body fluids fluoresce under specific wavelengths of light and can be more easily located with the aid of an alternate light source (ALS).

Note: When using an ALS, blood stains lack fluorescence and appear darker than the surrounding surfaces.

9.5.2 REAGENTS, CHEMICALS, STANDARDS, & CONTROLS

As no testing occurs during this visual assessment activity, there are no reagents, chemicals, or controls involved.

STANDARDS

- ALS Performance Verification Standard-Known Semen Standard

  SEMEN STANDARD FOR VERIFICATION OF ALTERNATE LIGHT SOURCES

  Dried preparations of both neat and dilute semen standards used to verify the performance of ALS equipment will be assigned a unique laboratory lot number given as SEM-ALS-YY##, whereas SEM indicates a semen standard, ALS indicates its use, YY indicates the year, and ## indicates the number of semen standard prepared in that year (i.e., SEM-ALS-2101=1st standard prepared in 2021). Document in logbook.

  Generally, one standard will be verified for use per calendar year, but the expiration date may be extended if fluorescence is still exhibited by additional verification testing at the end of its expiration date. Extensions will be recorded in the logbook on the original verification form and standards re-labeled with new expiration date information. If the quality of fluorescence diminishes before reaching an expiration date, a new standard can be made for use following procedures above.

9.5.3 SAMPLE PREPARATION

9.5.3.1 EVIDENCE ASSESSMENT

Visually examine the item for possible biological material. The ALS may be used when no visible seminal stains are noted.

9.5.3.2 SAMPLING METHOD

As ALS Examination can be considered another method of visual examination, the stains marked are also selected based on nonstatistical sampling.

- Nonstatistical Sampling- stains of interest for further body fluid testing are marked during the visual examination of evidence items. This process is based on the analyst's training and experience. This sampling method only answers questions about the portion tested. There is no assumption of homogeneity of all stains on the evidence item[63].

9.5.3.3 SAMPLING RECORDS

As nonstatistical sampling is the only approach taken in sampling of evidence in the Serology Unit, the record of the identification of the sampling method is listed in this section. Case notes provide the other relevant information required:

[63] Example: Shirt with four stains. One stain is chosen to be tested based on the analyst's training and experience. The results of the sampled stain do not apply to the other untested stains.

- Date of sampling (case notes date(s))
- Identification of the person performing the sampling (analyst's name or supervised trainee, if applicable)
- Identification and description of sample (Item name, stain #)
- Diagram(s) identifying the sampling location (photographs or stain location descriptions in notes)
- Any deviation, addition, or exclusion from the sampling method and plan[64].

9.5.3.4 METHOD

Seminal fluid, saliva, sweat, & urine may fluoresce with use of the following combination of glasses and wavelengths:

- 450 nm – orange glasses
- 530 nm – red or orange glasses
- 485 nm – red or orange glasses

1) When in the laboratory, the alternate light source shall be used in a dark environment to best illuminate possible stains. If outside of the laboratory and the examination environment is not conducive to dark lighting, the analyst may use their discretion to determine appropriate lighting when using the ALS.
2) Systematically scan the light over the evidence looking for stains or other evidence that may be of forensic value. Some stains may require using the ALS at various angles and proximities to visualize. For example, some stains may be illuminated when using the light 12 inches away at a 90 degree angle, where as some may require an oblique angle at 1-2 inches away.
3) Seminal stains typically present with consistent fluorescence throughout the stain surface whereas urine and saliva stains typically present with a concentrated "halo" of fluorescence around the edge of the stain with inner areas being less fluorescent. Sweat often presents with a general muted fluorescence without defined borders.
4) Circle or otherwise mark areas that fluoresce without marking on the stain so they may be located in normal lighting conditions for testing.
5) If no stains of interest are visible with the alternate light source, additional search methods (such as targeted swabbing, quadrant swabbing, regularly spaced cuttings) in conjunction with the appropriate testing methods (e.g., phenolphthalein, acid phosphatase testing, etc.) may be required for a thorough examination depending on the evidence, case, and other factors as determined by the analyst.

***Note*:** If the entire item shows no fluorescence, but the fabric pattern/construction is visually challenging using an ALS, (i.e., bright neon colors or vivid patterns), regardless of presumptive test

[64] Deviations from the sampling plan and procedures may be requested by the customer or deemed appropriate by the analyst. Any deviations shall be approved in writing by the appropriate Section Chief and maintained in the case record.

results (if general swabbing is used), a control cutting should generally be retained **if tapelifts have been retained**.

9.5.4 QUALITY ASSURANCE/CONTROL MEASURES

The Serology Unit Training Program includes training on proper ALS usage. Model differences, appropriate wavelength settings, and appropriate eyewear are taught as well as techniques for visually examining different types of evidence. It is the analyst's responsibility to verify that the appropriate wavelength is used in conjunction with the appropriate eyewear. Each time an ALS is used on an item or set of items, a performance check shall first be conducted using both neat and 1:5 dilutions of known semen samples to ensure fluorescence is observable. These samples are located in each scrape down room. Documentation of these checks shall be recorded by circling the appropriate PASS/FAIL area adjacent to the ALS number recorded on each page of semen examination notes. If an ALS unit fails a performance check, the equipment will be immediately taken out of service and the Forensic Serology Quality Manager notified in order to troubleshoot the cause of the failure. To proceed with testing, the analyst must add documentation of the ALS number of the alternate unit to be used as well as a successful performance check of that unit to their notes before beginning evidence testing.

***Note*:** If an analyst needs to use the same ALS later in the day, a new performance check shall be conducted and *additional* documentation of the ALS PASS/FAIL added near the beginning of the notes for that item or set of items.

9.5.5 INTERPRETATION OF RESULTS

9.5.5.1 PRECAUTIONS

- Semen stains will not always fluoresce; lack of fluorescence does not mean semen is not present (i.e., blood/semen mixtures).
- Other non-probative stains such as beverages, food products, mud or water stains, and detergents (especially whiteners) may fluoresce in a similar manner as body fluids and it may be difficult to distinguish stains of forensic interest from background fluorescence.
- Some brightly-colored fabrics (e.g., neon pink or orange) are challenging to examine due to the fabrics' strong fluorescent reaction when viewed with an ALS. In these situations, general swabbing (and subsequent presumptive chemical testing) of the probative areas for possible body fluid may be warranted (e.g., crotch of underwear) following ALS use. Documenting that nothing of value was noted for semen testing in these situations is not encouraged due to the fact that the visible examination of the item was limited.
- Never look directly into the light or allow beams to bounce off surfaces into your eyes or the eyes of other persons in the vicinity. Appropriate eyewear shall be worn to view evidence when using an alternate light source. Close scrape down room doors before using an ALS.

9.5.5.2 POSSIBLE SOURCES OF ERROR

- Incorrect glasses used: Depending on the ALS used, specific colored glasses must be used in conjunction with the wavelength setting in order to view fluorescence.
- Incorrect wavelength setting used: Some ALSs have optional wavelength settings or interchangeable heads. If the incorrect wavelength is used, body fluids may not fluoresce appropriately for detection.
- ALS is not brought close enough to the substrate for viewing: As the ALS approaches the surface being viewed, fluorescing stains will become more distinct. If starting with a weakly fluorescing stain, the fluorescence may be overlooked if the ALS is held too far away to view the item.
- ALS is used with normal lighting conditions: Dark conditions are necessary, whenever possible[65], to view fluorescence optimally with an ALS.

9.5.5.3 LITERATURE REFERENCES

- NFSTC – Testing of Body Fluids (Semen)
- *Forensic Biology*, 2nd Ed. Chapter 14

9.5.5.4 CRITERIA FOR RESULTS

The analyst must review all submitted information provided in a case to determine which types of stains may be present and which stains might be probative. If the alternate light source is used to identify possible semen stains, those stains are then tested to determine whether semen is present (see 9.4.3.4 for exceptions).

- If the alternate light source is used to identify stains of possible saliva, sweat, or urine that are considered probative to the case, then those stains are not typically tested for semen (unless mixtures are suspected), but are retained (e.g., bandana used as a facemask by a perpetrator fluoresces in an area that would be expected to contain saliva stains).

9.5.6 NOTES/DOCUMENTATION REQUIREMENTS

Analysts shall document in their notes when an alternate light source (ALS) is used to examine an item or set of items by filling in the ALS number. All applicable items of evidence listed on the same page of examination notes are understood to have been observed using the same ALS unless otherwise noted. Items that would not routinely be examined using an ALS (e.g., swabs) do not require any documentation of non-ALS usage.

Note: If an analyst uses a different ALS unit on any items within the same case, their notes must reflect the change of ALS number **and** performance check information (e.g., ALS-##: Pass/Fail) at the starting point of the notes for that specific item or set of items.

[65] Crime scenes may not always allow for completely dark conditions.

9.5.7 REPORT WRITING

See section 9.2.7 for general report writing information regarding Serology Autotext.

At the end of the visual examination process, the analyst will determine whether further testing is warranted for body fluid identification. If no further testing is required (i.e., no stains are located that require presumptive and/or confirmatory testing) a report may be created.

RESULTS CHART

The evidence item that was visually examined will be listed in the Serology Chart under the "Results" section of the report.

VISUAL EXAMINATION FOR SEMEN

Using JusticeTrax®, for each "NOVN Semen" item that was examined, the analyst will select "No Visual/ALS Stains" from the semen findings drop-down menu. This will populate the semen column on the report to read, "No Visual/ALS Stains."

Note: If an item is not tested for a particular body fluid, this may be reported by selecting "Not Tested" or "N/A" in the blood and/or semen findings drop-down menu(s).

If any stains require further serological testing, the report writing step will not occur until all of the testing has been completed.

9.5.8 CRITICAL REAGENTS & EQUIPMENT SPECIFICATIONS

There are no critical reagents or equipment used in the visual examination of stains using an ALS.

9.5.8.1 EQUIPMENT

ALTERNATE LIGHT SOURCE

1) Handling-Gloves are to be worn when handling the ALS. The ALS is considered biohazardous equipment. Analysts should take the necessary precautions to prevent hitting the ALS against surfaces or dropped as ALSs are susceptible to damage.
2) Transport- Outside of the laboratory, carrying cases (if provided) should be used when transporting the ALS and its accessories. If no case is provided, the analyst will secure the ALS to protect it from accidental drops, etc.
3) Storage-If ALSs are not going to be used for an extended period of time, they should be unplugged. Ideally, they should be stored inside cabinetry or covered to reduce dust exposure.
4) Use-ALSs are used to screen items of evidence for body fluids, such as semen or saliva.
5) Planned Maintenance-Annually cleaned and documented in the *Instrument Maintenance and Temperature Log* (SER-FORM-30) Binder.

9.6 ACID PHOSPHATASE TEST FOR THE PRESUMPTIVE SCREENING OF SUSPECTED SEMINAL STAINS

9.6.1 SCOPE

Seminal acid phosphatase (AP) is detected in stains of seminal origin through its hydrolysis of 5-bromo-4-chloro-3-indolyl phosphate (BCIP) to an insoluble stable blue product. It is considered a presumptive test. AP is present in high quantities in comparison to other substances which makes it a useful screening test for semen.

9.6.2 REAGENTS, CHEMICALS, STANDARDS, & CONTROLS

REAGENTS

- BCIP: 5-bromo-4-chloro-3-indolyl phosphate [66]

CHEMICALS

- Sodium Acetate Buffer[67]

Christmas Tree Stains (See sections 9.8.2 and 9.8.8.1 for more information on stains.)

STANDARDS

- Reference Standard-Known Semen Standard

  SEMEN STANDARD FOR VERIFICATION OF ACCURACY OF DETECTION TESTS, REAGENTS, AND TECHNIQUES

  Dried semen standards used to verify the accuracy of detection tests, reagents, and techniques will be assigned a unique laboratory lot number given as SEM-YY##, whereas SEM indicates a semen standard, YY indicates the year, and ## indicates the number of semen standard prepared in that year. For example, the first semen standard prepared in the year 2012 would be given the unique laboratory lot number of SEM-1201. Document in logbook with the case number of the sample.

  There will be one semen standard verified for use per calendar year. This standard may be divided for use in different Forensic Serology laboratory areas, if needed. When necessary, additional standard(s) may be verified throughout the year if the original standard fails to maintain compliance.

[66] See Serology Reagent Prep. Logbook or Qualtrax® for preparation information.
[67] See Serology Reagent Prep. Logbook or Qualtrax® for preparation information.

The source of the standard will be documented in the Reagent Logbook. However, if the standard is collected from an individual and that person wishes to remain anonymous or is unknown, a general designation of the source may be used instead.

The sample will be subjected to the tests, reagents, and techniques for which it is used as a positive control in casework and an accurate positive result must be obtained. The date of this verification will be recorded along with the initials of the person performing the verification, date sample was collected, and a description of the standard preparation. The expiration date of a semen standard shall be one year, and it may be extended by subjecting the sample to additional verification testing at the end of its expiration date. This information will be recorded in the Reagent Logbook on form.

Dried semen standards may be stored in the refrigerator or at room temperature. The documentation located in the Reagent Logbook will be maintained in the Forensic Serology Unit.

CONTROLS

NEGATIVE CONTROL FOR VERIFICATION OF ACCURACY OF DETECTION TESTS, REAGENTS, AND TECHNIQUES

In the Serology Unit, a negative control is used with each testing method. This can be either a sterile swab or unadulterated reagent (known as a reagent blank). The sample will be subjected to the tests and reagents for which it is used as a negative control in casework. An accurate negative result must be obtained to ensure the tests and reagents are working properly. These results will be recorded in the appropriate locations, as outlined in each testing method.

The following controls are to be used with each run. A run is considered to be a set of stains in separate test tubes that will undergo acid phosphatase testing in a water bath. A run oftentimes includes swabs from different items of evidence. There shall be appropriate substrate controls present to represent each item of evidence in a run. Results of control testing will be recorded in the analyst's case notes.

- **Reagent blank (negative control)** – a sterile swab is placed directly in a labeled tube. BCIP is added.
- **Positive control** – a moistened swab applied to a known semen standard and placed directly in a labeled tube. BCIP is added.
- **Substrate Control**: a moistened swab is applied to an unstained area on the questioned item and placed directly in a labeled tube. (See section 9.2.2 for additional information.)

Note: For small items such as genital wipes, if the entire item fluoresces using an ALS, a control does not need to be tested or collected. If a non-fluorescing area is identified, proceed with Substrate Control testing (see above) and retain the control if any samples for DNA potential (cuttings or tapelifts) are retained from the wipe.

Note: Sanitary pads/napkins and tampons are not required to be tested for blood. Proceed with this section's methods (for sanitary pads/napkins) if semen testing is warranted. Refer to Section 9.14.2 if a tampon is submitted.

9.6.3 SAMPLE PREPARATION

9.6.3.1 EVIDENCE ASSESSMENT

After stains have been visually located, use a test tube rack to place labeled glass tubes in order based on Q# and stain number. Also place labeled control tubes (See section 9.6.2) in the rack.

Note: Swabs with suspected semen stains are normally handled by following section 9.7 Extraction of Suspected Semen Stains protocol which involves cutting a portion of each swab into a centrifuge tube and soaking in the appropriate buffer. However, conducting Acid Phosphatase testing on the swabs is an acceptable method as well. Transferring stain from a submitted swab to a test swab can occasionally present challenges, especially if the submitted swab was not dampened adequately by the medical staff or law enforcement agency that collected it. Care should be taken to ensure adequate swabbing of the questioned swab transfers the suspected semen stain to the dampened test swab.

Sexual Assault Kit swabs are typically not serologically tested at all but rather portions are cut into DNA extraction tubes (See section 9.13.1). The Physical Evidence Section Chief will inform the Serology Unit analysts if the handling guidelines/procedures for these types of samples change to a more classical approach in which they would be serologically tested based on communications with the Forensic DNA Section and/or ASCL Administrators.

9.6.3.2 METHOD

1) Sample a questioned stain by rubbing with a cotton-tipped applicator moistened with distilled water. Place swab in a labeled test tube.
2) Once all swabs are collected, add adequate BCIP reagent to each tube to cover the tips of the swabs, and incubate test tube rack in water-bath at an approximate temperature of 37˚± 5˚C for 15 minutes.

Note: To avoid potential contamination of the BCIP stock solution, use a clean pipette to obtain each *additional* aliquot of reagent that may be needed to fill any remaining test tubes. Avoiding contact of pipette tips with the test tube rims and swab sticks is also recommended.

3) After incubation, acid phosphatase activity is indicated by the development of a blue to aqua-blue color on the swabs.

DAB SLIDE TECHNIQUE

1) A strongly positive swab from the AP presumptive screening test may be used to make a microscope slide for identification of spermatozoa by dabbing or rubbing the positive swab onto a properly labeled microscope slide.
2) Dry slide in oven and proceed with the Christmas Tree Stain for Identification of Spermatozoa method (See section 9.8).

Note: If (+) for spermatozoa, document slide results in a way that makes it clear a dab slide was used to represent the entire stain[68] instead of preparing a slide from a single cutting of the stain.

3) If no spermatozoa are identified, the analyst shall return to the evidence and proceed with the regular Extraction of Suspected Semen Stains method.

9.6.4 QUALITY ASSURANCE/CONTROL MEASURES

Controls (listed in section 9.6.2) are used with each run to ensure that a quality testing process is achieved. The reagent blank (negative control) ensures there are no deficiencies with the BCIP reagent being used. The positive control ensures that the BCIP is functioning properly and produces an expected positive AP reaction (blue color change). The substrate control tests an unstained area of the evidence to detect whether there are substances intrinsic to the evidence which may be exhibiting acid phosphatase activity.

As with all Serology Unit methodology, using clean techniques when conducting acid phosphatase testing on potential semen stains is imperative to the procedure. The Serology Training Program covers all aspects of the proper techniques to sample and test stains with BCIP. On-the-job training and experience are the most important factors in developing and refining effective testing skills.

9.6.5 INTERPRETATION OF RESULTS

9.6.5.1 PRECAUTIONS

- Proper precautions shall be taken to ensure that each swab and test tube used does not make contact with other swabs and/or test tubes in the run.
- Before adding BCIP to the tubes, visualize the reagent to ensure that it is clear. Over time, blue tints may form. Expiration dates have been put in place to prevent this, but it is the analyst's responsibility to assess the solution before using it.
- Inadequate or excessive dampening of the swabs with distilled water can prevent proper transfer of stain to the swab tips and therefore potentially lead to non-representative test results.

[68] Positive Dab Slide results represent spermatozoa quantities from the entire swabbed surface area of the semen stain. Subsequent cuttings from that stain which are submitted to the DNA Section for extraction may or may not reflect a lower quantity of foreign male DNA. Documentation of using the Dab Slide technique helps to explain any quantitative differences between the sections in the laboratory.

9.6.5.2 POSSIBLE SOURCES OF ERROR

- Some surfaces may contain bacteria or other enzymatically active substances which may produce a positive result. While the color is typically not the characteristic aqua blue seen with seminal acid phosphatase and may be more blueish-green, it can be misleading. The analyst will use their training and experience to determine if further testing is needed, but should continue with confirmatory testing if there is a possibility that semen may be present.
- Inadequate pressure may prevent transfer of stain to the test swab and therefore produce misleading results.
- BCIP doesn't cover the swab tip in the test tube. If adequate reagent isn't in contact with the sampled stain, chemical reactions may not occur.
- Too much time passes between sampling a stain and conducting the AP test. Sample degradation can occur over time, therefore incorrect results may be obtained if the testing is delayed. See section 9.6.5.5 for more information.

9.6.5.3 LITERATURE REFERENCES

- NFSTC – Testing of Body Fluids (Semen)
- *Forensic Biology*, 2nd Ed. Chapter 14

9.6.5.4 CRITERIA FOR RESULTS

- Positive Result: Aqua blue color change is observed on the swab. The blue/aqua-blue BCIP hydrolysis product is indicative of acid phosphatase activity and is recorded as a positive (+) result. Proceed to confirmatory testing procedures for semen. If the positive result is an *intense* blue/aqua blue color, the analyst may opt to use the Dab Slide Technique at this point (see section 9.6.3.2 Dab Slide Technique).
- ***Note***: It is not always necessary to perform confirmatory testing on all positive BCIP stains on an item. The analyst will use his or her own judgment and experience to determine when further testing is not necessary. For example, if a bed sheet has ten stains with positive BCIP results and the analyst has identified sperm cells on one or more of those stains, then the analyst may describe and retain the remaining BCIP positive stains without performing confirmatory tests for semen.Negative Result: No color change occurs. No color change indicates the absence of acid phosphatase and is recorded as a negative (-) result. No further testing is required.

9.6.5.5 TROUBLESHOOTING

1) **Reagent blank (Negative control)** produces a positive result. Notify Physical Evidence Section Chief of nonconformity in testing. Document result in case notes.
   a) Obtain a new reagent blank and retest all samples.
      - If testing of new reagent blank yields negative results, discard previous (old) reagent blank and record findings.

- If testing of new reagent blank yields positive results then make new BCIP reagent.
- Verify BCIP reagent by retesting old and new reagent blanks along with a positive control and recording in the Reagent Logbook.
- If testing of reagent blanks yield negative results, then discard old BCIP reagent and record findings appropriately.

b) Start a Quality Assurance Concern workflow as necessary.

2) **Positive control** produces a negative result. Notify Physical Evidence Section Chief of nonconformity in testing. Document result in case notes.
   a) Obtain a new positive control and retest all samples.
      - If testing of new positive control yields positive results, discard previous (old) positive control and record findings.
      - If testing of new positive control yields negative results then make new BCIP reagent.
      - Verify BCIP reagent by retesting old and new positive controls along with a reagent blank and recording in the Reagent Logbook.
      - If testing of positive control yield positive results then discard old BCIP reagent and record findings appropriately.
   b) Start a Quality Assurance Concern workflow as necessary.

3) **Substrate Control** produces a positive result.
   a) Retest by selecting a different area to use as the substrate control.
      - If negative, record all test results in notes, use the tested area with negative results as the substrate control site, and retain as necessary.
      - If positive, record all test results in notes, select either tested area to use as the substrate control, and retain as necessary.
   b) It is equally acceptable to document in notes that the control area which tested positive exhibited no fluorescence and opt not to test a different area as a substrate control. Retain the substrate control as necessary.

   Note: some surfaces may contain bacterial content or other enzymatically active substances which may produce a positive result.
   c) If there is no other suitable area to test for a control, record test results in notes and retain as necessary.
4) **Test swabs**
   a) Swabs can be oversaturated with distilled water. This can prevent adequate transfer of the stain to the swab for testing.
   b) Inadequate pressure during sample collection will result in insufficient sample being transferred to the swab.
   c) Degradation of sample can occur when test swabs are allowed to stand too long between stain sampling and reagent addition. A maximum of two hours is allowable between test swab collection and reagent addition.

9.6.6 NOTES/DOCUMENTATION REQUIREMENTS

1) The results obtained from the testing of questioned stains and substrate controls are recorded in the notes.
2) The results of the positive and negative controls are recorded in the notes.
3) The analyst will check the temperature of the water bath at each use to verify that the temperature is within the specified temperature range of 37˚C ± 5˚C. This pass/fail verification is recorded in the notes.

Note: If the stain is too limited in size to conduct presumptive tests without the risk of total consumption of the sample by testing, the abbreviation "QNS" (quantity not sufficient) may be written in the appropriate test column. If the presumptive test is positive but the stain is too limited in size to conduct confirmatory tests without the risk of total consumption of the sample by testing, the abbreviation "QNS" may be written in the appropriate test column(s). This approach is sometimes necessary to conserve the limited stain sample for further processing in the Forensic DNA section at the expense of opting not to identify the body fluid contained in the questioned stain.

9.6.7 REPORT WRITING

See section 9.2.7 for general report writing information regarding Serology Autotext.

- **Positive**: DO NOT REPORT- Proceed to confirmatory testing.
- Positive, but not enough sample for confirmatory testing: "Indicated*" may be listed in the semen column of the report. In the "Further Explanation of Results" section of the report, "*Presumptive tests for the presence of semen were positive on Q#; confirmatory tests were not conducted due to limited sample quantity" can be written. (See QNS note under section 9.6.6 Notes/Documentation Requirements.)
- **Negative**: A negative result signifies that no semen was chemically identified. If all stains that are presumptively tested on an evidence item are negative, the analyst will select "Negative" in the semen findings drop-down meu.

9.6.8 CRITICAL REAGENTS & EQUIPMENT SPECIFICATIONS

There are no critical reagents or equipment used in the AP test for the presumptive screening of suspected seminal stains.

9.6.8.1 EQUIPMENT

DISPOSABLE PIPETS

a) Handling-Gloves shall be worn when handling pipets in conjunction with casework. If new aliquots of BCIP are required to fill all test tubes, a new pipet shall be used to prevent

possible contamination of the stock solution. Any remaining BCIP in a pipet should not be added back to the BCIP stock solution bottle.

b) Transport-Pipets are available at most work areas in the laboratory, but if they are transported, the analyst should guard against any possible contamination.
c) Storage-Pipets shall be stored in clean conditions and protected from exposure to liquids or evidence.
d) Use-Pipets are used to dispense BCIP solution into test tubes in this method. Pipets are disposable and shall not be reused but rather disposed of after use.
e) Planned Maintenance-Not Applicable

REFERENCE MATERIAL – SEMEN STANDARD

a) Handling- Semen Standards are enclosed in a labeled petri dish. Gloves shall be worn when handling. Semen Standards are considered to be biohazardous equipment.
b) Transport- If transported, petri dish lid should be closed to prevent accidental contamination of surfaces. Standard should be transported in a manner that will prevent accidental drops.
c) Storage-Maintained in a dried condition at room temperature typically, but may be refrigerated.
d) Use-Positive Control for AP testing procedure; sampled with a dampened sterile swab for testing.
e) Planned Maintenance-one semen standard is prepared annually. However, if the AP reaction becomes faint prematurely, a new standard may be made.

REAGENT – BCIP (WORKING SOLUTION)

a) Handling-Gloves and lab coat shall be worn when handling.
b) Transport-typically is not subjected to transport, but if bottle is removed some distance from the laboratory refrigerator, the analyst shall ensure that the cap is secured and take precautions to prevent accidental drops of the glass container.
c) Storage-under refrigeration when not in use
d) Use-reagent that facilitates observation of AP reaction (SER-FORM-13).
e) Planned Maintenance-Unless the solution begins to turn blue prior to the expiration date, new BCIP is prepared every 3 months. It shall not be used beyond its labeled expiration date.

CHEMICAL–SODIUM ACETATE BUFFER

a) Handling-Gloves and lab coat shall be worn when handling.
b) Transport- typically is only subjected to transport when preparing the working solution of BCIP. If bottle is removed some distance from the laboratory refrigerator, the analyst shall ensure that the cap is secured and take precautions to prevent accidental drops of the glass container.
c) Storage-under refrigeration when not in use.

d) Use-Acetate Buffer is used in the preparation of the working solution of BCIP (SER-FORM-12).
e) Planned Maintenance-Acetate Buffer is generally made in such a quantity that more than one batch of BCIP working solution can be made. It shall not be used beyond its labeled expiration date.

CHEMICAL–1% ACETIC ACID

a) Handling-Gloves and lab coat shall be worn when handling.
b) Transport- if transported, the analyst shall ensure that the cap is secured and take precautions to prevent accidental drops of the glass container.
c) Storage-room temperature
d) Use-1% acetic acid is used to adjust the pH of the Acetate Buffer.
e) Planned Maintenance-there is no formal planned maintenance of the acid. If it is found to lose its effectiveness in adjusting the pH, it shall be properly disposed of and new acetic acid prepared (SER-FORM-20).

CHEMICAL–CHRISTMAS TREE STAINS

See sections 9.8.2 and 9.8.8.1 for more information on stains.

PH METER

a) Handling-Gloves and lab coat shall be worn when handling.
b) Transport- if transported, use the provided carrying case. The analyst shall ensure that the case latches are secured and take precautions to prevent accidental drops of the pH meter.
c) Storage-carrying case, room temperature
d) Use-to measure pH of the Acetate Buffer when 1% acetic acid is used for adjustments.
e) Planned Maintenance-the pH meter is cleaned annually. Analysts will ensure that upon each use, the electrode buffer level is checked and documented in the *Instrument Maintenance and Temperature Log* (SER-FORM-30) Binder. If necessary, electrode buffer will be added. A performance check will also be performed with each use and recorded on the *Serology pH Meter Calibration Log* (SER-FORM-33).

REFRIGERATOR

a) Handling-Gloves shall be worn when accessing the refrigerator as it is considered biohazardous equipment.
b) Transport-Not Applicable, but if necessary, should be unplugged and moved immediately OR allowed to thaw if storage is imminent.
c) Storage-Not Applicable, but if necessary, should be unplugged and allowed to thaw/dry completely to prevent mold/mildew.
d) Use-refrigeration of BCIP reagent.

e) Planned Maintenance-Temperatures are checked monthly and refrigerators are annually cleaned and documented in the *Instrument Maintenance and Temperature Log* (SER-FORM-30) Binder.

WATER BATH

a) Handling- Water Baths are considered biohazardous equipment. Gloves shall be worn when handling them.
b) Transport-normally stationary, but if transport is required, turn power off, remove the thermometer, and drain of water to prevent accidental spills. Once it is relocated and refilled, the temperature shall be checked and documented in the *Instrument Maintenance and Temperature Log* (SER-FORM-30) Binder.
c) Storage-Lids shall be closed when water baths are not in use to deter evaporation.
d) Use-used to facilitate the AP reaction for presumptive testing via heated water. The analyst shall verify that adequate water is present and at a correct range of temperature.
e) Planned Maintenance- Temperatures are checked with each use as well as monthly and water baths are annually cleaned and documented in the *Instrument Maintenance and Temperature Log* (SER-FORM-30) Binder. Water is added on an as-needed basis due to evaporation.

GLASSWARE (FOR REAGENT PREPARATION)

a) Handling-Gloves should be worn when handling glassware.
b) Transport-if transported, the analyst will take the proper precautions to guard against breaking or dropping glassware.
c) Storage-Glassware will be stored in a clean, dry condition and readily available for the next use.
d) Use-Glassware is used when preparing reagents. See the Reagent Prep Logbook for detailed instructions.
e) Planned Maintenance-Glassware is visually checked before use to ensure that it is not chipped or faulty. No other maintenance is required, other than expected cleaning at the end of each use.

ANALYTICAL BALANCE (FOR REAGENT PREPARATION)

a) Handling-as analytical balances are sensitive equipment, they should be handled carefully and minimally. Gloves and lab coat are recommended to be worn when using an analytical balance.
b) Transport-analytical balances should not be moved, ideally. If transport is needed, contact the DNA Section Quality Manager for assistance on reestablishing “in service” use.
c) Storage-when not in use, analytical balances will remain uncovered and in clean condition on the bench top of the Serology prep area. If extended storage is required, consult the DNA section for guidance.

d) Use-The analytical balance is used to weigh out powders for the preparation reagents and/or chemicals.
e) Planned Maintenance-The analytical balance is maintained by the Forensic DNA Section. Analysts using the balance are expected to maintain its cleanliness and remove any residual powders from surfaces between uses.

9.7 EXTRACTION OF SUSPECTED SEMEN STAINS FOR ANALYSIS OF SOLUBLE AND PARTICULATE SEMINAL COMPONENTS

9.7.1 SCOPE

Sperm cells and/or p30 (sometimes called PSA) are accepted markers for detecting semen; detection is accomplished through microscopic or chemical examination of the extract.

9.7.2 REAGENTS, CHEMICALS, STANDARDS, & CONTROLS

REAGENTS

- Not applicable

CHEMICALS

- SERATEC®-provided PBS or HEPES

STANDARDS

- Not applicable

CONTROLS

- Not applicable

9.7.3 SAMPLE PREPARATION

9.7.3.1 METHOD

The following procedure will provide an extract of the soluble substances and a pellet of the particulate material for analysis.

1) Place cuttings of questioned stain (approximately 2.5 mm^2 for cloth; approximately ¼ of a cotton-tipped applicator) into a graduated micro centrifuge tube or well-top micro centrifuge tube.
2) Fill the tube to the 1.0 mL mark with SERATEC®-provided PBS buffer (or HEPES) for each sample, and allow two hours for extraction at room temperature. Extraction may also be

accomplished overnight under refrigeration. (If using non-graduated micro centrifuge tubes, add approximately 1 mL (or ~17 drops using disposable pipette) of buffer.)

Note: If at this point adequate time does not remain to prepare the microscope slide(s) on the same day, it is recommended to store micro centrifuge tubes (may be refrigerated or frozen overnight) *prior to* centrifugation, as the cell button or pellet may be disturbed upon retrieval, necessitating a second centrifugation. If the sample is frozen at any point after the addition of buffer but before the required 2 hours of extraction time has passed, the analyst shall allow 2 hours of extraction time, in total, to pass while the buffer is in a thawed condition.

3) Centrifuge to maximize recovery of extract and pellet.
   a) Using a clean wooden applicator or similar device, agitate the cutting, remove cutting from tube, and place in a Spin-X® tube filter basket. Place the basket into the top of the micro centrifuge tube and secure lid. This allows as much fluid as possible to be extracted from the sample.

Note: If using a well-top micro centrifuge tube, the cap must be perforated with a dissecting probe or similar tool. The sample cutting will then be placed in the closed well-top cap before centrifugation.

Caution: The centrifuge must be balanced with a blank 1 mL water-filled centrifuge tube if the samples to be extracted aren't in an even numbered quantity.

   b) Centrifuge approximately three minutes.
   c) Remove and properly dispose of the Spin-X® basket containing the sample cutting in the appropriate biohazard waste container. The micro centrifuge tube now contains the supernatant, which is ready for SERATEC® PSA Semiquant testing, and a pellet of particulate material, which may contain sperm cells.
4) Preparation of Slide from Pellet
   a) Separate pellet from supernatant by removing supernatant with a micropipette to a second labeled centrifuge tube OR by removing the pellet with a micropipette and dispensing onto a labeled microscope slide while leaving the supernatant in the original tube.
   b) If pellet is in original tube after step 4a, use micropipette to break up pellet and pipet onto a labeled microscope slide.

   Note: If a large pellet is observed, additional buffer may be added at this step to dilute the cellular sample, which will improve visibility of the sample on the microscope slide.
   c) Dry slide in oven and proceed with Christmas Tree staining procedure (see section 9.8).

9.7.4 QUALITY ASSURANCE/CONTROL MEASURES

As with all Serology Unit methodology, using clean techniques when conducting extraction of suspected semen stains for analysis of soluble and particulate seminal components is imperative to

the procedure. The Serology Training Program covers all aspects of the proper techniques to extract stains.

9.7.5 INTERPRETATION OF RESULTS

As this is an extraction step only, there are no results to interpret.

9.7.5.1 PRECAUTIONS

- Proper cutting size of stains is imperative to achieve reliable test results after extraction.
- Adequate buffer should be used in conjunction with a 2 hour soaking time to facilitate proper extraction.
- If centrifuge tube top well requires perforation, ensure there are enough adequately sized holes to allow for supernatant and cellular material to travel to the base of the tube but small enough to maintain the cutting in the well. Otherwise, fibers may become incorporated in the cell button and can obscure the analyst's field of view while screening the microscope slide that will be prepared.
- To prevent cross-contamination, use a clean wooden applicator stick for the retrieval of each stain sample.
- To prevent cross-contamination, use a clean, disposable micropipette to separate each tube's supernatant from its cell button.

9.7.5.2 POSSIBLE SOURCES OF ERROR

The analyst is responsible for properly labeling each centrifuge tube and ensuring that the correct cutting is placed in the matching tube. Otherwise, extractions can become associated with the incorrect stain, leading to incorrect interpretation upon further test methods.

9.7.5.3 LITERATURE REFERENCES

- SERATEC® PSA Semiquant product insert
- NFSTC – Testing of Body Fluids (Semen)

9.7.5.4 CRITERIA FOR RESULTS

As this is an extraction step only, there are no criteria for results.

9.7.6 NOTES/DOCUMENTATION REQUIREMENTS

No additional notes or documentation are required at this step.

9.7.7 REPORT WRITING

A report will not be written at this step. Continue with section 9.8 steps.

9.7.8 CRITICAL REAGENTS & EQUIPMENT SPECIFICATIONS

There are no critical reagents or equipment used in this extraction step.

9.7.8.1 EQUIPMENT

CHEMICALS – SERATEC®–PROVIDED PBS OR HEPES

a) Handling-Gloves and lab coat shall be worn when handling buffers.
b) Transport-when transported, ensure the cap is secured. If HEPES is used, precautions should be taken so as to not drop the glass container.
c) Storage-PBS buffer and HEPES buffer are stored in the refrigerator when not in use. They may be left out of the refrigerator during working hours.
d) Use-buffer is used to extract soluble substances and particulate material from substrates.
e) Planned Maintenance-none, however expiration dates shall be observed. Once PBS has been successfully QCd, it may be used with future Lot #s of QCd SERATEC® microcassettes but ONLY IF it is within its expiration date. If using HEPES, it may continue to be used until it reaches its expiration date.

REFRIGERATOR

a) Handling-See section 9.6.8.1.
b) Transport- See section 9.6.8.1.
c) Storage- See section 9.6.8.1.
d) Use-refrigeration of buffer(s).
e) Planned Maintenance- See section 9.6.8.1.

DISPOSABLE PIPETS

a) Handling- See section 9.6.8.1.
b) Transport- See section 9.6.8.1.
c) Storage-See section 9.6.8.1.
d) Use-for dispensing buffer into the centrifuge tubes containing stain samples. Pipets are disposable and shall be disposed of after use.
e) Planned Maintenance-Not Applicable

GLASSWARE (FOR REAGENT PREPARATION)

a) Handling-See section 9.6.8.1.
b) Transport- See section 9.6.8.1.
c) Storage- See section 9.6.8.1.
d) Use-Glassware is used when preparing HEPES buffer. See the Reagent Prep Logbook for detailed instructions.
e) Planned Maintenance- See section 9.6.8.1.

ANALYTICAL BALANCE (FOR REAGENT PREPARATION)

a) Handling- See section 9.6.8.1.
b) Transport- See section 9.6.8.1.
c) Storage- See section 9.6.8.1.
d) Use-The analytical balance is used to weigh out powders for the preparation of HEPES.
e) Planned Maintenance- See section 9.6.8.1.

PH METER

a) Handling- See section 9.6.8.1.
b) Transport- See section 9.6.8.1.
c) Storage- See section 9.6.8.1.
d) Use-to measure pH of the HEPES for adjustments.
e) Planned Maintenance- See section 9.6.8.1.

CENTRIFUGE

a) Handling-Gloves shall be worn when handling the centrifuge. It is considered biohazardous equipment.
b) Transport-normally stationary, but if transport is required, close lid, unplug, and carry in such a way that safeguards against drops.
c) Storage- unplug and store with the lid closed.
d) Use-to separate cellular components of semen stains from the soluble components (supernatant).
e) Planned Maintenance-centrifuges are annually cleaned and documented in the *Instrument Maintenance and Temperature Log* (SER-FORM-30) Binder.

9.8 CHRISTMAS TREE STAIN FOR MICROSCOPIC IDENTIFICATION OF SPERMATOZOA

9.8.1 SCOPE

The Christmas Tree staining method is the most reliable microscopic visual confirmation for the presence of sperm cells. Picroindigocarmine (PICS) stains the tail portion of the sperm cell green, and Nuclear Fast Red stains the head of the sperm cell red.

9.8.2 REAGENTS, CHEMICALS, STANDARDS, & CONTROLS

REAGENTS

- Not applicable

CHEMICALS

- Picroindigocarmine (PICS) stain
- Nuclear Fast Red stain
- Xylene
- Permount™

STANDARDS

- Not applicable

CONTROLS

- Not applicable

9.8.3 SAMPLE PREPARATION

9.8.3.1 METHOD

1) Pipet cell button onto a clean, labeled[69] microscope slide.

***Note*:** While not mandatory, analysts may use a wax pencil to demarcate a central area of the slide inside of which the cell button may be pipetted. This allows for easier location of the edges of the stained area.

2) Dry slide in oven.
3) Cover stain in 100% ethanol and allow cell button to dry in oven.
4) Add a few drops of Nuclear Fast Red stain to slide. Allow to sit approximately 15–20 minutes[70].
5) GENTLY wash off stain with distilled water.
6) Dry slide in oven.
7) Add PICS stain and then rinse with 100% ethanol after approximately 10-15 seconds[71].
8) Dry slide in oven.
9) Use Permount™ sparingly if applying a cover slip. Excess mounting medium may be cleaned with Xylene in a fume hood.

***Note*:** Permount™ is stamped with an expiration date by the manufacturer; however, this mounting medium may be used past the expiration date as long as it is checked to ensure proper quality. A successful QC performance check extends the expiration date 1 year. This QC performance check is recorded on the Permount™ QC Worksheet in the Reagent Logbook.

[69] Label minimally with the laboratory case #, item #, and analyst's initials
[70] Nuclear Fast Red stain can be left on a slide for longer than 15 minutes, but the analyst should ensure that the stain is not allowed to completely dry, as concentrated stain is difficult to rinse off and can partially obscure the visibility of the cellular material on the slide.
[71] PICS stain is an intense and fast-acting cellular stain. The analyst should ensure that the stain is not left in contact with the slide for more than approximately 15 seconds. Cellular material can easily stain too darkly and visibility can be greatly reduced.

10) Examine slides using microscope (suggested screening objective 20X; suggested confirmation objective 40X).
11) Using a dotting pen or other appropriate writing instrument, mark the slide near spermatozoa. Marking a representative quantity of cells is recommended. If multiple spermatozoa are present, dotting is not necessary.

9.8.4 QUALITY ASSURANCE/CONTROL MEASURES

***Note*:** For approximately one month after an analyst completes the Serology training program, all negative sperm slides in their casework shall be reviewed by another qualified analyst, with the reviewer initialing the negative results on the semen examination form. If the date is different than the date at the top of the examination form, the date will be recorded by the reviewer's initials.

SLIDE DISPOSITION

1) Another qualified analyst MUST confirm all positive identifications of sperm cells.
2) All slides prepared by the analyst must contain the laboratory case number, item number, and analyst initials. If the slides do not contain a "frosted" area upon which to write, a diamond-tip applicator may be used to write on the slide itself.
3) When verifying sperm cells, the confirming analyst must ensure the laboratory case number and corresponding item number on each slide matches the laboratory number of the analyst's notes.
4) Positive slides made from evidence material will typically be returned with the evidence, or positive slides may be retained for submission to the DNA section.
5) All negative slides made from evidence will be discarded appropriately.
6) Though not routine, if Medical Examiner slides are submitted to the Forensic Serology Unit, they will be returned to the Medical Examiner's Section after examination.

9.8.5 INTERPRETATION OF RESULTS

9.8.5.1 PRECAUTIONS

Morphological differences between spermatozoa and other cellular debris on slides can be challenging. Analysts should approach the screening of slides attentively and carefully. Conservative decisions should be kept in mind when yeast cells (especially budding presentations) or epithelial nuclei stripped of their cytoplasm are present. Both can mimic spermatozoa nuclei. However, attributes such as size, acrosome absence, staining intensity, and background will allow the analyst to discern appropriately between the cell types.

Proper preparation of the specimen is key to appropriate interpretation. As mentioned above, too thick of a cell button or inadequate/overabundant staining can interfere with visibility.

Microscopes should be in proper alignment and maintained for optimal screening quality. Though serviced annually, analysts are responsible for the upkeep of their microscopes. Cleaning and alignment procedures (including Köhler Illumination instructions) can be found on the S: drive>Microscopes folder.

9.8.5.2 POSSIBLE SOURCES OF ERROR

Although the Serology Training Program allows for ample opportunity to gain the proper knowledge and experience needed for correctly screening and identifying spermatozoa, it is a subjective task. Opinions may differ and if there is any question upon initial screening of a specimen, the analyst is encouraged to consult a qualified analyst for a second opinion on dotted areas of challenging slides.

See section 9.8.5.1 for additional information on possible sources of error.

9.8.5.3 LITERATURE REFERENCES

- NFSTC – Testing of Body Fluids (Semen)
- *Forensic Biology*, 2nd Ed. Chapter 14

9.8.5.4 CRITERIA FOR RESULTS

At a minimum, the posterior head with the anterior acrosome present is required for positive identification of a sperm cell.

- Sperm Head—Anterior Head (Acrosome) = light red/clear
- Sperm—Posterior Head = dark red
- Sperm—Mid-piece (mitochondrial sheath) = green
- Sperm—Tail (flagellum) = green
- Epithelial cells—Nucleus = light red
- Epithelial cells—Cytoplasm = light green

9.8.6 NOTES/DOCUMENTATION REQUIREMENTS

1) If sperm cells are identified, record positive results in the case notes. Another qualified analyst will confirm the positive identification of sperm cells; this confirmation is recorded in the case notes. Describe the stain(s) in the case notes. A thorough description will include the location of the stain or a drawing showing the location of the stain and a measurement of the stain. Photographs may substitute for the description of the stain(s).
   a) The analyst may choose to use a scale ranging from "+" to "+++" to indicate the approximate number of sperm cells identified on the slide.
   +++: Numerous sperm cells are present in every field-of-view of the slide.
   ++: Several sperm cells are present in most fields-of-view of the slide.
   +: Sperm cells are present, but not in the quantity as described for "++" or "+++."

b) The analyst may also make additional notes if necessary (i.e., tails present or (1) sperm cell observed.) If a DAB slide technique was used, this should be recorded in the notes for that stain.
c) If sperm cells are identified on a sample, no further semen testing is necessary for that stain sample. The sample is retained using a collection method as outlined in section 9.3 Collection of Stains for Further Testing.

2) If no sperm cells are found, record negative results in the case notes.
3) If no sperm cells are found, proceed to p30 testing (see section 9.9).

9.8.7 REPORT WRITING

If no sperm cells are identified, semen testing is not complete at this step and the analyst will proceed to p30 testing (see section 9.9) before writing a report.

See section 9.2.7 for general report writing information regarding Serology Autotext.

RESULTS CHART

The evidence item(s) from which stains were examined microscopically for spermatozoa and found to be positive will be listed in the Serology Chart under the "Results" section of the report.

REPORT FINDINGS

Using JusticeTrax®, for each (+) spermatozoa evidence item that was examined, the analyst will select "Sperm Cell(s) Identified" in the semen findings drop-down menu. This will populate the semen column on the report to read "Sperm Cell(s) Identified."

RETAINED SAMPLES

Under the Retained Samples portion of the report, the analyst will use the appropriate retained samples listing(s) below:

- **Retained Items**: The appropriate Q#(s) will be listed on the "Item(s)…" line.
- **Retained Cuttings**: The appropriate Q#(s) will be listed on the "Cutting(s) from…" line.
- **Retained Swabs**: The appropriate Q#(s) will be listed on the "Swab(s) from…" line.

9.8.8 CRITICAL REAGENTS & EQUIPMENT SPECIFICATIONS

There are no critical reagents or equipment used in the staining and microscopic identification of spermatozoa.

9.8.8.1 EQUIPMENT

CHEMICALS

- Ethanol
    a) Handling- gloves shall be worn when handling ethanol bottles.

b) Transport- ensure cap is in place to prevent unexpected dispensing of ethanol.
c) Storage-store with cap securely fastened to prevent evaporation.
d) Use-ethanol is used to fix the cell button stain sample onto the microscope slide.
e) Planned Maintenance-none, no expiration date

- Picroindigocarmine (PICS) stain
  a) Handling-gloves shall be worn when handling stain bottles.
  b) Transport-ensure cap is in place to prevent unexpected dispensing of stain.
  c) Storage-store with cap securely fastened to prevent evaporation and blockage of dispenser tip.
  d) Use-for staining cellular components on microscope slide preparations.
  e) Planned Maintenance-none, however expiration dates shall be observed.

- Nuclear Fast Red stain
  a) Handling-gloves shall be worn when handling stain bottles.
  b) Transport-ensure cap is in place to prevent unexpected dispensing of stain.
  c) Storage-store with cap securely fastened to prevent evaporation and blockage of dispenser tip.
  d) Use-for staining cellular components on microscope slide preparations.
  e) Planned Maintenance-none, however expiration dates shall be observed.

- Permount™
  a) Handling-gloves shall be worn, fume hood blowers turned on, and fume hood sashes lowered appropriately when handling Permount™.
  b) Transport-ensure cap is in place and proper precautions are taken to prevent accidental spillage and/or drops of glass bottle container.
  c) Storage-the cap should always be closed when not in use as Permount™ can lose viscosity upon exposure to air. Due to flammability, while small, capped bottles are permitted to remain in hoods, bulk stock shall be stored in the flammables cabinet.
  d) Use-as a mounting media for adhering cover slips to microscope slides.
  e) Planned Maintenance-no maintenance is required, however expiration dates shall be observed. (See "Note" below).

  Caution: Analysts that are pregnant should not use Permount™ as it can be dangerous to the unborn child.

  ***Note*:** Permount™ is stamped with an expiration date by the manufacturer; however, this noncritical mounting medium may be used past the expiration date as long as it is checked to ensure proper quality is preserved. A successful QC performance check extends the expiration date one year. This QC performance check is recorded on the Permount™ QC Worksheet in the Reagent Logbook.

- Xylene

a) Handling-gloves shall be worn, fume hood blowers turned on, and fume hood sashes lowered appropriately when dispensing Xylene.
b) Transport- ensure cap is in place and proper precautions are taken to prevent accidental spillage and/or drops of glass bottle container.
c) Storage-due to flammability, Xylene will be stored in the flammables cabinet (bulk stock) and in fume hoods (working stock, capped when not in use).
d) Use-for cleaning of excess Permount™ on microscope slides and also for occasional thinning of Permount™ to maintain adequate viscosity.
e) Planned Maintenance-none, no expiration date.

Caution: Xylene is a known carcinogen and teratogen. Breathing vapors should be avoided. Analysts that are pregnant should not use Xylene as it can be dangerous to the unborn child.

DUCTLESS HOOD

a) Handling-control panel buttons are considered clean. If wearing gloves, they must be clean.
b) Transport-N/A.
c) Storage-if taking hood out of use, unplug power cord and ensure sash is lowered.
d) Use-for coverslip application and analyst protection from chemical fumes.
e) Planned Maintenance-Hoods are annually cleaned and documented in the *Instrument Maintenance and Temperature Log* (SER-FORM-30) Binder. Calibration and filter changes are performed and recorded as needed.

MICROSCOPE

a) Handling-Microscopes are considered clean equipment. Gloves shall **not** be worn when using them. Analysts shall handle microscopes with care. Do not look into the light. Be cautious when handling slides. It is recommended to both place and remove slides while the scope is set to the 10X objective to prevent accidental lens contact with the specimen.
b) Transport- To prevent damage to the microscope and to guard against injury, always carry the microscope with two hands. Do not touch the lens.
c) Storage-When not in use, microscopes should be protected with a dust cover.
d) Use-In this method, microscopes are used to screen prepared slides for spermatozoa. Analysts will verify that the bulb is functioning properly and the correct objectives are being used to both screen and confirm slides.
e) Planned Maintenance-Microscopes are cleaned annually and documented in the *Instrument Maintenance and Temperature Log* (SER-FORM-30) Binder. Analysts may perform Köhler Illumination steps as needed to optimize screening quality (see S: Drive >Microscopes folder for instructions).

DISPOSABLE MICROPIPETS

a) Handling-Gloves shall be worn when handling micropipets in conjunction with casework.
b) Transport-micropipets are available at most work areas in the laboratory, but if they are transported, the analyst should guard against any possible contamination.

c) Storage-micropipets shall be stored in clean conditions and protected from exposure to liquids or evidence.
d) Use-micropipets are used to deposit cell button contents onto labeled microscope slides. Micropipets are disposable and shall be disposed of after use. One micropipet shall be used per stain specimen to prevent contamination between stains.
e) Planned Maintenance-Not Applicable

OVEN

a) Handling-Gloves shall be worn when handling the oven. Ovens are considered biohazardous equipment.
b) Transport-Not Applicable, but if necessary, should be turned off, unplugged, and allowed to cool first.
c) Storage-if necessary, should be turned off, unplugged, and allowed to cool first.
d) Use-ovens are used to expedite the drying of specimens on microscope slides as well as expedition of the fixing process of the specimen with ethanol.
e) Planned Maintenance-ovens are cleaned annually and documented in the *Instrument Maintenance and Temperature Log* (SER-FORM-30) Binder.

9.9 IDENTIFICATION OF SEMEN USING THE SERATEC® PSA SEMIQUANT

9.9.1 SCOPE

The SERATEC® PSA Semiquant test is designed to identify p30 (sometimes called prostate-specific antigen or PSA) qualitatively for the forensic identification of semen. P30 is an acceptable confirmatory marker for the identification of semen. Its main function is to liquefy the seminal fluid.

Note: Reported p30 concentrations in semen range from 200,000 ng/mL to 5.5 million ng/mL. Reported p30 concentrations in other body fluids are *significantly* lower (e.g., amniotic fluid ~0.60-8.98 ng/mL, breast milk ~0.47-100 ng/mL, etc.) The test method utilized by the ASCL Serology Unit provides confidence in the origin of any p30 being identified in casework as prostatic due to purposeful selection of controlled sample size and dilution factors.

9.9.2 REAGENTS, CHEMICALS, STANDARDS, & CONTROLS

REAGENTS

- Not applicable

CHEMICALS

- SERATEC®-provided Phosphate Buffered Saline (PBS)
- HEPES

STANDARDS

- Reference Material-Known Semen Standard (for QC of new lot # of SERATEC® cards only) See 9.6.2 for additional information.

CONTROLS

The following control testing shall be conducted once per day when SERATEC® PSA Semiquant cards are to be used in the laboratory[72].

- **Reagent blank (negative control)** –buffer is added to a labeled centrifuge tube. The result of the daily negative control is recorded in the case notes by all analysts using the SERATEC® product/buffer that was QCd that day[73]. A positive reaction of a negative control renders the test invalid. See section 9.6.2 for additional information about reagent blanks.
- **Positive control -** a positive control using a cutting of a known semen standard and buffer is added to a labeled centrifuge tube. This control is only required to be performed when new lot numbers are obtained. All results are recorded on the SERATEC® Card Quality Assurance Worksheet in the Reagent Logbook and are recorded in the case notes by all analysts using the SERATEC® product that day.

9.9.3 SAMPLE PREPARATION

9.9.3.1 METHOD

1) Allow sample (supernatant from section 9.7 Extraction of Suspected Semen Stains for Analysis of Soluble and Particulate Seminal Components) and negative control buffer (when used) to warm to room temperature. SERATEC® cards should be stored in the refrigerator until day of use; allow SERATEC® cards to warm to room temperature before use.
2) Remove SERATEC® card and dropper from sealed pouch.
3) Label SERATEC® cards with the appropriate item number and case number. Only one p30 test is performed per sample.
4) Add approximately 5 drops (~200µL) with the supplied micropipette of the supernatant from the extract to the sample well.

Note: Should the sample inadvertently be deposited in the sample well along with the extract and buffer, the sample will not interfere with the test results.

5) Read results at 10 minutes. Results past 10 minutes are not valid.

[72] The same Lot # of Seratec® PSA cards and same bottle of buffer must be used for one negative control to satisfy the QC requirements per work day.
[73] If more than one bottle of buffer is being used, a separate daily negative control is required for each bottle of buffer. If more than one Lot # of Seratec® PSA cards are being used, a separate daily negative control is required per Lot #.

9.9.4 QUALITY ASSURANCE/CONTROL MEASURES

9.9.4.1 TROUBLESHOOTING

1) Positive Control produces a negative result during quality assurance testing. Notify Physical Evidence Section Chief of nonconformity in testing. Document result in case notes.
   a) Obtain a new positive control and retest using a new test cassette.
      - If testing of new positive control yields positive results, discard previous (old) positive control and record findings.
      - If testing of new positive control yields negative results then obtain new SERATEC®-provided PBS buffer (or make new HEPES buffer solution).
      - Verify SERATEC®-provided PBS buffer (or HEPES) by retesting old and new positive controls along with a reagent blank and recording in the Reagent Logbook.
      - If testing of positive control yields positive results then discard old SERATEC®-provided PBS buffer (or HEPES) and record findings appropriately.
   b) Start a Quality Assurance Concern workflow as necessary.
2) Negative Control (reagent blank) produces a positive result. Notify Physical Evidence Section Chief of nonconformity in testing. Document result in case notes.
   a) Select approximately five new test cassettes from the current lot and test with SERATEC®-provided PBS buffer (or HEPES) only.
      - If testing yields negative results then record findings.
      - If testing yields positive results then obtain new SERATEC®-provided PBS buffer (or make new HEPES buffer).
      - Verify SERATEC®-provided PBS buffer (or HEPES buffer) by selecting two to five cassettes from each remaining box and retesting along with a positive control and recording in the Reagent Logbook.
      - If testing of reagent blanks yield negative results then discard SERATEC®-provided PBS buffer (or old HEPES buffer) and record findings appropriately.
   b) Start a Quality Assurance Concern workflow as necessary.
3) **Invalid result**: Internal standard line and or control line are not detectable **OR** test line is not complete[74]. Notify the Physical Evidence Section Chief of nonconformity in testing. Document result in case notes.
   a) Repeat assay with a new test cassette.
   b) **Cautions:**
   - A specimen pH value below 3 or above 12 SU can cause false or invalid results.
   - A high viscosity of the sample might interfere with the capillary flow.
   - Do not use test cassettes or the SERATEC®-provided PBS buffer after the expiration date or if the pouch has been damaged.
   c) Start a Quality Assurance Concern workflow as necessary.

[74] Horizontal test line is partial, blurred, etc.

Note: Exposure to heat may negatively affect the integrity and reliability of the SERATEC® cards. Refrigeration when not in use is a preventative measure employed by the Serology Unit. However, if unexpected power outages affect the temperature of the storage environment, caution should be used when affected lot #s of cards are used after such an event, if known. A new lot # may need to be obtained if the daily QC does not pass.

9.9.5 INTERPRETATION OF RESULTS

9.9.5.1 PRECAUTIONS

Caution: Though sexual assault swabs collected during the course of an autopsy typically are submitted directly to the Forensic DNA section, it is important to recognize that if testing is conducted by the Serology Unit at any stage, deceased males may test (+) p30 on rectal swabs that are collected postmortem due to tissue degradation. If p30 testing is conducted on this sample type in the Forensic Serology Unit, clear communication with the customer(s) is encouraged in order to explain the limits on interpretation from such samples. Additional Forensic DNA testing on the rectal swabs may resolve questions, but is not guaranteed.

The SERATEC® cards are manufactured with areas of pink dye-labeled antibodies. These areas of the card may appear shadowed when supernatant wicks across that zone. Analysts should only interpret the test area as being positive if actual pink dye is observed (regardless of intensity.) Brighter lit areas of the laboratory may aid in correct interpretation of these areas of the card.

9.9.5.2 POSSIBLE SOURCES OF ERROR

See section 9.9.4.1 for possible sources of error, generally.

In addition, the analyst is responsible for appropriate sampling and dilution of the stains to be tested using the SERATEC® product. (See also section 9.9.5.4, part 2(b).)

9.9.5.3 LITERATURE REFERENCES

- SERATEC® PSA Semiquant product insert
- *Forensic Biology*, 2nd Ed. Chapter 14
- NFSTC – Testing of Body Fluids (Semen)

9.9.5.4 CRITERIA FOR RESULTS

1) Positive Results: Three pink lines indicate the test result is positive: one in the test area (T), one in the control area (C), and one internal standard line. According to the SERATEC® instructions, “any visible T-line (strong or weak colored) indicates a positive result.
2) Negative Results: Two pink lines indicate a negative test: one line in the control area (C) and one internal standard line.

A negative result indicates either:

a) No detectable p30 is present.
b) Sample was not properly diluted. This can cause the presence of a "High Dose Hook Effect," which may give false negative results due to the presence of high concentration of p30 in a sample (i.e., undiluted/neat seminal fluid). However, if proper procedures are followed, (all samples are diluted using 1 mL of SERATEC®-provided PBS buffer (or HEPES)) the high dose hook effect is not encountered in the Serology Unit of the ASCL and therefore, does not lead to false negative results. Seminal fluid tests positive in the dilution range from 1:1 to 1:10 using SERATEC® PSA Semiquant.

3) Invalid: No pink line visible in the control area or no internal standard line visible **OR** test line is not complete renders the test invalid.

9.9.6 NOTES/DOCUMENTATION REQUIREMENTS

1) If the p30 tests are positive, record the positive results in the notes. Describe the stain(s) in the case notes. A thorough description will include the location of the stain or a drawing showing the location of the stain and a measurement of the stain. (scaled photos can substitute for this info.)
2) If the p30 test is negative, record the negative result in the notes.
3) Describe the stain(s) in the case notes. A thorough description will include the location of the stain or a drawing showing the location of the stain and a measurement of the stain.
4) The results of the positive and negative QC controls are recorded in the notes.
5) The SERATEC® cassette test Lot# is recorded in the notes.

9.9.6.1 ASSESSMENT OF RESULTS

- If the SERATEC® test is positive, then the analyst can report that semen was identified on that item. The sample is then retained using a collection method as outlined in section 9.3 Collection of Stains for Further Testing.
- If the SERATEC® test is negative, then the analyst can report that no semen was found on that item.

9.9.7 REPORT WRITING

See section 9.2.7 for general report writing information regarding Serology Autotext.

RESULTS CHART

The evidence item(s) from which stains were tested for p30 and found to be positive will be listed in the Serology Chart under the "Results" section of the report.

REPORT FINDINGS

Using JusticeTrax®, for each (+) p30 evidence item that was examined, the analyst will select "p30 Identified" in the semen findings drop-down menu. This will populate the semen column on the report to read "p30 Identified."

Note: A "p30 Identified" result signifies that no sperm cells were found, but p30 (a component of semen) was identified. A "Negative" result signifies that no semen was chemically identified.

RETAINED SAMPLES

Under the Retained Samples portion of the report, the analyst will use the appropriate retained samples listing(s) below:

- **Retained Items**: The appropriate Q#(s) will be listed on the "Item(s)…" line.
- **Retained Cuttings**: The appropriate Q#(s) will be listed on the "Cutting(s) from…" line.
- **Retained Swabs**: The appropriate Q#(s) will be listed on the "Swab(s) from…" line.

9.9.8 CRITICAL REAGENTS & EQUIPMENT SPECIFICATIONS

There are no critical reagents used in the identification of semen using the SERATEC® PSA Semiquant as a confirmatory test for the presence of semen.

9.9.8.1 CRITICAL EQUIPMENT

SERATEC® PSA SEMIQUANT

a) Handling- Gloves shall be worn when handling SERATEC® PSA Semiquant.
b) Transport-If transporting cards, best practice is to leave the pouches sealed until reaching the testing location.
c) Storage- SERATEC® PSA Semiquant cards shall be stored in the refrigerator overnight
d) Use- SERATEC® PSA Semiquant cards are used as a confirmatory test for the presence of semen.
e) Planned Maintenance-none, however expiration dates shall be observed.

9.9.8.2 EQUIPMENT

REFRIGERATOR

a) Handling-See section 9.6.8.1.
b) Transport-See section 9.6.8.1.
c) Storage- section 9.6.8.1.
d) Use-for refrigeration of SERATEC® PSA Semiquant cards and buffer(s).
e) Planned Maintenance- section 9.6.8.1.

9.10 PHENOLPHTHALEIN TEST FOR THE PRESUMPTIVE SCREENING OF SUSPECTED BLOOD STAINS

9.10.1 SCOPE

This oxidative test for the presumptive identification of blood is based on catalytic activity of the heme group of hemoglobin. The Phenolphthalein test is also known as the Kastle Meyer test. It is not specific to blood only.

9.10.2 REAGENTS, CHEMICALS, STANDARDS, & CONTROLS

REAGENTS

- Phenolphthalin (reduced form of phenolphthalein)

CHEMICALS

- 3% hydrogen peroxide

STANDARDS

- Known Blood Standard

  BLOOD STANDARD FOR VERIFICATION OF ACCURACY OF DETECTION TESTS, REAGENTS, AND TECHNIQUES

  Dried blood standards used to verify the accuracy of detection tests, reagents, and techniques will be assigned a unique laboratory lot number given as BLD-YY##, whereas BLD indicates a blood standard, YY indicates the year, and ## indicates the number of blood standard prepared in that year. For example, the first blood standard prepared in the year 2012 would be given the unique laboratory lot number of BLD-1201.

  The donor of the source will be identified, however if an individual donor wishes to remain anonymous or is unknown, a general designation of the source may be used instead.

  The sample will be subjected to the tests, reagents, and techniques for which it is used as a positive control in casework and an accurate positive result must be obtained. The date of this verification will be recorded along with the initials of the person performing the verification, date sample was collected, and a description of the standard preparation. The expiration date of a blood standard shall be one year, and it may be extended by subjecting the sample to additional verification testing at the end of its expiration date. This information will be recorded in the Reagent Logbook.

  Dried blood standards may be stored in either the refrigerator or the freezer. The documentation located in the Reagent Logbook will be maintained in the Forensic Serology Unit.

CONTROLS

- **Positive Control**: A known blood sample is tested using the method described below. The test is considered positive as indicated in section 9.10.5 Interpretation of Results below.
- **Negative Control**: A negative control is tested using the method described below. A negative control sample is a sterile, unstained cotton-tipped applicator, which is designated a negative control sample. See section 9.6.2 for additional information.
- **Substrate Control**: A moistened swab is applied to an unstained area on the questioned item and is tested using the method described below.

Results of control testing will be recorded in the analyst's case notes.

9.10.3 SAMPLE PREPARATION

9.10.3.1 METHOD

1) Sample a questioned stain by lightly rubbing with a cotton-tipped applicator moistened with distilled water, ensuring visible transfer of stain to moistened swab.
2) Add 1-2 drops of phenolphthalin (clear) to the swab and observe for detection of any oxidative contaminants that may be present.
3) Add 1-2 drops 3% hydrogen peroxide and carefully observe to detect any pink color (phenolphthalein form), which usually develops immediately. Do not observe results beyond ~10 seconds.

Caution: Do not touch dropper tips to swabs during testing to prevent potential contamination of working solutions.

Note: Sanitary pads/napkins and tampons are not required to be tested for blood.

9.10.4 QUALITY ASSURANCE/CONTROL MEASURES

As with all Serology Unit methodology, using clean techniques when conducting the phenolphthalein test on possible bloodstains is imperative to the procedure. The Serology Training Program covers all aspects of the proper techniques to test possible bloodstains.

Daily positive and negative QC checks are performed separately on any sets of phenolpthalin and 3% HOOH that are to be used on casework. This ensures that the reagent and chemical are working properly as well as demonstrates the absence of contamination in the solutions.

9.10.5 INTERPRETATION OF RESULTS

9.10.5.1 PRECAUTIONS

Phenolphthalin is a highly sensitive reagent. However, it is not highly specific and therefore is considered a presumptive test. Any substances that have the ability to act as a chemical oxidant may yield a positive reaction. Some examples: potatoes, horseradish, cabbage, copper and nickel salts, etc.

In the absence of blood, the two reagents will begin to react due to environmental oxidation and develop a hot pink color with time. For this reason, do not observe any results beyond ~10 seconds.

9.10.5.2 POSSIBLE SOURCES OF ERROR

Adequate transfer of questioned stain to test swab was not achieved. This may be due to over-or under-dampening the cotton-tipped applicator with distilled water. The analyst should always visualize the test swab after sampling to verify transfer of stain is visibly confirmed.

Application of reagent and chemicals to the test swab was not achieved. The analyst should visualize the swab tip as they are dispensing both phenolphthalin and 3% HOOH to be confident that all necessary solutions have been deposited on the swab tip with the questioned stain.

Touching the dispenser tips to the questioned stain. While this possible error will not impact the current stain being tested, it may lead the analyst to interpret false positive results on future tested stains due to contamination of the reagents and/or chemicals involved.

9.10.5.3 LITERATURE REFERENCES

- NFSTC – Testing of Body Fluids (Blood)
- *Forensic Biology*, 2nd Ed. Chapter 2, 12, & 13

9.10.5.4 CRITERIA FOR RESULTS

1) Positive Reaction will show a strong hot pink color within approximately 5 seconds following the addition of the hydrogen peroxide.

Caution: Do not interpret test results beyond ~10 seconds, as a positive pink result will eventually occur on all swabs due to environmental oxidation.

2) Negative Reaction will not have an instant hot pink color.

9.10.5.5 TROUBLESHOOTING

1) **Positive Control** yields a negative result. Notify Physical Evidence Section Chief of nonconformity in testing. Document in case notes.
   a) Obtain a new positive control and retest.

- If testing of new positive control yields positive results, discard previous (old) positive control and record findings.
- If testing of new positive control yields negative results then obtain new phenolphthalin working solution.
- Verify phenolphthalin working solution by retesting old and new positive controls along with a reagent blank and recording in the Reagent Logbook.
- If testing of positive control yield positive results, then discard old phenolphthalin working solution and record findings appropriately.

b) Start a Quality Assurance Concern workflow as necessary.

2) **Negative Control** yields a positive result. Notify Physical Evidence Section Chief of nonconformity of testing. Document in case notes.
   a) Obtain a new reagent blank and retest.
      - If testing of new reagent blank yields negative results, discard previous (old) reagent blank and record findings.
      - If testing of new reagent blank yields positive results, then obtain new phenolphthalin working solution.
      - Verify phenolphthalin working solution by retesting old and new reagent blanks along with a positive control and recording in the Reagent Logbook.
      - If testing of reagent blanks yield negative results then discard old phenolphthalin working solution and record findings appropriately.
   b) Start a Quality Assurance Concern workflow as necessary.
3) **Substrate control** produces a positive result.
   a) Retest by selecting a different area to use as the substrate control.
      - If negative, record all test results in notes, use the tested area with negative results as the substrate control site, and retain as necessary.
      - If positive, record all test results in notes, select either tested area to use as the substrate control, and retain as necessary.
   b) If there is no other suitable area to test for a control, record test results in notes and retain as necessary.

9.10.6 NOTES/DOCUMENTATION REQUIREMENTS

1) The results obtained from testing of questioned stains and substrate controls are recorded in the case notes.
2) The results of the positive and negative controls are recorded in the case notes.
3) The Lot # of the phenolpthalin reagent is recorded in the case notes.

Note: If the stain is too limited in size to conduct presumptive tests without the risk of total consumption of the sample by testing, the abbreviation "QNS" (quantity not sufficient) may be written in the appropriate test column. If the presumptive test is positive but the stain is too limited in size to conduct confirmatory tests without the risk of total consumption of the sample by testing, the abbreviation "QNS" may be written in the appropriate test column(s). This approach is

sometimes necessary to conserve the limited stain sample for further processing in the Forensic DNA section at the expense of opting not to identify the body fluid contained in the questioned stain.

9.10.6.1 ASSESSMENT OF RESULTS

- Positive Result: Hot pink color change is observed. Proceed to confirmatory testing procedures for blood.
- Negative Result: No pink color change is observed. No further testing is required.

9.10.7 REPORT WRITING

- **Positive**: DO NOT REPORT—Proceed to confirmatory testing.
- Positive, but not enough sample for confirmatory testing: "Indicated*" may be listed in the blood column of the report. In the "Further Explanation of Results" section of the report, "*Presumptive tests for the presence of blood were positive on Q#; confirmatory tests were not conducted due to limited sample quantity" can be written. (See QNS note under section 9.10.6. Notes/Documentation Requirements.)
- **Negative**: A "Negative" result signifies that no blood was chemically identified.
- "See Below*" may be listed in the blood column of the report if presumptive tests could not be conducted due to limited sample quantity. In the "Further Explanation of Results" section of the report, "*Q(#) was not tested for the presence of blood due to limited sample quantity" can be written. (See QNS note under section 9.10.6. Notes/Documentation Requirements.)
- See section 9.2.7 for general report writing information regarding Serology Autotext.

RESULTS CHART

- The evidence item(s) from which stains were presumptively tested for blood and found to be negative will be listed in the Serology Chart under the "Results" section of the report.

REPORT FINDINGS

- Using JusticeTrax®, for each negative (-) evidence item that was examined, the analyst will select "Negative" in the blood findings drop-down menu. This will populate the blood column on the report to read "Negative."

9.10.8 CRITICAL REAGENTS & EQUIPMENT SPECIFICATIONS

There are no critical reagents or equipment used in the Phenolphthalein Test for the Presumptive Screening of Suspected Blood Stains.

9.10.8.1 EQUIPMENT

REAGENTS

- Phenolphthalin (reduced form of phenolphthalein)
    a) Handling- gloves shall be worn when handling phenolpthalin bottles.
    b) Transport- ensure cap is in place to prevent unexpected dispensing of phenolpthalin. Carry securely to avoid potential breakage of glass bottle.
    c) Storage-store in amber bottle with cap securely fastened to prevent deterioration or loss.
    d) Use-phenolphthalin is used as a reagent in the test method to detect the presumptive presence of blood.
    e) Planned Maintenance-expiration dates will be followed. Dropper should be maintained in clean condition. If buildup occurs, clean as needed to prevent premature oxidation of the reagent.

CHEMICALS

- 3% hydrogen peroxide
    a) Handling-gloves shall be worn when handling hydrogen peroxide bottles.
    b) Transport- ensure cap is in place to prevent unexpected dispensing of hydrogen peroxide. Carry securely to avoid potential breakage of glass bottle.
    c) Storage – store with cap securely fastened to prevent loss.
    d) Use- 3% hydrogen peroxide is used as a chemical in the test method to detect the presumptive presence of blood.
    e) Planned Maintenance-expiration dates will be followed. Dropper should be maintained in clean condition.

REFRIGERATOR

a) Handling-See section 9.6.8.1.
b) Transport- section 9.6.8.1.
c) Storage- section 9.6.8.1.
d) Use-refrigeration of phenolphthalin and 3% HOOH when not in use
e) Planned Maintenance- section 9.6.8.1.

9.11 TAKAYAMA REAGENT AS A CONFIRMATORY TEST FOR BLOOD

9.11.1 SCOPE

Confirmation of the presence of the heme group in hemoglobin in suspected bloodstains is accomplished by the development and microscopic identification of water-insoluble crystals, known as pyridine ferroprotoporphyrin crystals. Takayama is specific to blood, but cannot

differentiate between different sources (e.g., animal blood or human blood). Also referred to as the Hemochromogen Test.

9.11.2 REAGENTS, CHEMICALS, STANDARDS, & CONTROLS

REAGENTS

- Takayama

CHEMICALS

- Pyridine
- 10% NaOH
- Saturated Glucose

STANDARDS

- Known Blood Standard (daily QC use only) See section 9.10.2 for additional information.

CONTROLS

- **Positive Control**: Reagent reliability is checked daily prior to use in casework by testing a positive control of known blood.
- **Negative Control**: Reagent reliability is checked daily prior to use in casework by testing a negative control of a sterile swab. See section 9.6.2 for additional information.

Results of control testing will be recorded in the analyst's case notes.

9.11.3 SAMPLE PREPARATION

9.11.3.1 METHOD

1) Place a small portion (thread, scraping, cutting, etc.) of the suspected stain on a slide and cover with a fragment of a coverslip.
2) Using a dropper, apply the Takayama reagent to the microscope slide adjacent to the coverslip. Allow reagent to flow under the coverslip, covering the questioned material. Avoid excess reagent.
3) if needed, place slide on slide warmer or in oven (~70 to 80˚C for approximately 20–30 seconds). The development of a pink color in and around the sample usually accompanies the reaction.

9.11.4 QUALITY ASSURANCE/CONTROL MEASURES

As with all Serology Unit methodology, using clean techniques when conducting the Takayama test on possible bloodstains is imperative to the procedure. The Serology Training Program covers all aspects of the proper techniques to test possible bloodstains.

Daily positive and negative QC checks are performed on any bottles of Takayama reagent that are to be used on casework. This ensures that the reagent is working properly as well as demonstrates the absence of contamination in the solution.

9.11.5 INTERPRETATION OF RESULTS

9.11.5.1 PRECAUTIONS

- Do not allow the dropper tip to come into contact with the slide, coverslip, or sample to prevent possible contamination of the Takayama Reagent.
- Leaving the slide in the oven or on the slide warmer too long will cause the Takayama Reagent to dry and darken to a degree that the sample is no longer useful for interpretation. If this occurs, it is recommended to prepare a new slide with a new sample from the stain in question.
- Caution should be used when rotating objectives. Some specimens may cause the cover slip to angle upward on the microscope slide. Contact between the objective and coverslip should be avoided.

9.11.5.2 POSSIBLE SOURCES OF ERROR

- Inadequate sampling of questioned stain. If too small of a sample is placed on the slide, the concentration of blood may be too limited to exhibit a positive reaction.
- Inadequate application of Takayama reagent. If too little Takayama is added to the sample, adequate saturation may not occur, impeding the chemical reactions necessary to view a positive reaction.
- Samples placed too closely together on a microscope slide. While it is acceptable to place more than one stain sample on a slide for testing, the analyst should ensure adequate space is maintained between the stains. Otherwise, Takayama solution may flow from one stain into the zone of a second stain, thus contaminating its test results.

9.11.5.3 LITERATURE REFERENCES

- NFSTC – Testing of Body Fluids (Blood)
- *Forensic Biology,* 2nd Ed. Chapter 2, 12, & 13

9.11.5.4 CRITERIA FOR RESULTS

1) A positive test is indicated by the observation of pink, feathery crystals of pyridine ferroprotoporphyrin. Use of 100X magnification is usually adequate but higher magnification may be helpful in some cases.
    a) Formation of pyridine ferroprotoporphyrin (Takayama crystals) confirms the presence of heme, and hence blood, in the stain.

b) Both high magnification and focusing to view through the depth of the sample are often helpful to locate small crystals or those that may be hidden within the fibers of a fabric sample.
c) Extended periods of time may be required after heating to allow for crystal development. Short time periods (5–10 minutes) of refrigeration at ~4˚C or freezing at ~ (-)20˚C may also enhance crystal development.
Note: Oily substrates may not allow the Takayama reagent to interact with the substrate adequately, thus inhibiting a reaction. If no crystals are visible, but oily globules are present microscopically, it is recommended that the analyst documents this visual observation and proceeds to an additional confirmatory test (i.e., HemaTrace®).
d) Crystal morphology may be altered slightly in decomposing blood; morphology may more resemble plates and/or needles.

2) A negative test is indicated by the absence of pink feathery crystals of pyridine ferroprotoporphyrin.

9.11.5.5 TROUBLESHOOTING

1) **Positive Control** yields a negative result. Notify Physical Evidence Section Chief of nonconformity in testing. Document in case notes.
   a) Obtain a new positive control and retest.
      - If testing of new positive control yields positive results, discard previous (old) positive control and record findings.
      - If testing of new positive control yields negative results then make new Takayama solution.
      - Verify Takayama working solution by retesting old and new positive controls along with a reagent blank and recording in the Reagent Logbook.
      - If testing of positive control yield positive results then discard old Takayama reagent and record findings appropriately.
   b) Start a Quality Assurance Concern workflow as necessary.
2) **Negative Control** yields a positive result. Notify Physical Evidence Section Chief of nonconformity of testing. Document in case notes.
   a) Obtain a new reagent blank and retest.
      - If testing of new reagent blank yields negative results, discard previous (old) reagent blank and record findings.
      - If testing of new reagent blank yields positive results then make new Takayama reagent.
      - Verify Takayama reagent by retesting old and new reagent blanks along with a positive control and recording in the Reagent Logbook.
      - If testing of reagent blanks yield negative results, then discard old Takayama reagent and record findings appropriately.
   b) Start a Quality Assurance Concern workflow as necessary.

9.11.6 NOTES/DOCUMENTATON REQUIREMENTS

1) If the Takayama test is positive, record the positive result in the case notes. Describe the stain in the case notes. A thorough description will include the location of the stain or a drawing showing the location of the stain and a measurement of the stain.
2) If the Takayama test does not yield the expected morphology, record the negative result in the case notes.
3) The results of the positive and negative controls are recorded in the case notes along with the Lot # of the Takayama reagent.

9.11.6.1 ASSESSMENT OF RESULTS

a) If the Takayama test is positive, then the analyst can report that blood was identified on that item. The sample is then retained using a collection method as outlined in section 9.3 Collection of Stains for Further Testing.
b) If the Takayama test is negative, then the analyst may report that no blood was chemically identified on that item. However, if the analyst suspects that a component of the sample may have inhibited crystal formation, they should proceed to HemaTrace® testing of that item and report results accordingly.

9.11.7 REPORT WRITING

- Positive: A "+" result signifies that blood was identified.
- If blood testing was negative using the Takayama blood test, proceed to HemaTrace® testing before reporting test results for that item.

RESULTS CHART

- The evidence item(s) from which stains were tested for blood with Takayama and found to be positive will be listed in the Serology Chart under the "Results" section of the report.

REPORT FINDINGS

- Using JusticeTrax®, for each positive evidence item that was examined, the analyst will select "Identified" in the blood findings drop-down menu. This will populate the blood column on the report to read "Identified."

RETAINED SAMPLES

Under the Retained Samples portion of the report, the analyst will use the appropriate retained samples listing(s) below:

- **Retained Items**: The appropriate Q#(s) will be listed on the "Item(s)…" line.
- **Retained Cuttings**: The appropriate Q#(s) will be listed on the "Cutting(s) from…" line.
- **Retained Swabs**: The appropriate Q#(s) will be listed on the "Swab(s) from…" line.

9.11.8 CRITICAL REAGENTS & EQUIPMENT SPECIFICATIONS

There are no critical reagents or equipment used in the Takayama Test as a confirmatory test for blood.

9.11.8.1 EQUIPMENT

REAGENTS

- Takayama
    a) Handling-Gloves shall be worn when handling Takayama bottles.
    b) Transport-Takayama is stored in amber glass stoppered bottles and should be transported with the cap sealed, in such a way to guard against accidental drops.
    c) Storage- Takayama may be stored in a sealed bottle at room temperature for 7 days OR in a freezer for 1 month.
    d) Use-Takayama is used as a confirmatory test for the identification of blood.
    e) Planned Maintenance-none, however expiration dates (based on chosen storage option) shall be observed.

CHEMICALS

- Pyridine
    a) Handling-Gloves shall be worn when handling Pyridine. Use a venting hood when bottle is uncapped.
    b) Transport-Pyridine is stored in an amber glass bottle and should be transported with the cap sealed, in such a way to guard against accidental drops.
    c) Storage-Pyridine is flammable and shall be stored in the flammables cabinet when not in use.
    d) Use-in the preparation of Takayama solution
    e) Planned Maintenance-none

- 10% NaOH
    a) Handling-Gloves shall be worn when handling 10% NaOH.
    b) Transport-10% NaOH is stored in a glass bottle and should be transported with the cap sealed, in such a way to guard against accidental drops.
    c) Storage-when not in use, 10% NaOH is stored in the hood when not in use.
    d) Use-in the preparation of Takayama solution
    e) Planned Maintenance-none, however expiration dates shall be observed.

DUCTLESS HOOD

a) Handling-See section 9.8.8.1.
b) Transport-See section 9.8.8.1.
c) Storage- See section 9.8.8.1.
d) Use- analyst protection from chemical fumes during Takayama preparation.

e) Planned maintenance- See section 9.8.8.1.

MICROSCOPE

a) Handling-See section 9.8.8.1.
b) Transport- See section 9.8.8.1.
c) Storage- section 9.8.8.1.
d) Use-Microscopes are used to view the microscopic crystals that are formed by the Takayama reaction with blood. Analysts should verify that the bulb is functioning properly and the correct objectives are being used to screen and confirm slides.
e) Planned Maintenance- section 9.8.8.1.

OVEN

f) Handling-See section 9.8.8.1.
g) Transport-See section 9.8.8.1.
h) Storage- See section 9.8.8.1.
i) Use-ovens are used to optionally expedite the reaction of the Takayama reagent (especially if newly prepared or from a frozen preparation) with blood.
j) Planned maintenance- See section 9.8.8.1.

VIEW BOX

a) Handling-Gloves shall be worn when handling the view box. View boxes are considered biohazardous equipment.
b) Transport-Not Applicable, but if necessary, should be turned off, unplugged, and allowed to cool first.
c) Storage-if necessary, should be turned off, unplugged, and allowed to cool first.
d) Use-view boxes are used to optionally expedite the reaction of the Takayama reagent (especially if newly prepared or from a frozen preparation) with blood.
e) Planned Maintenance-view boxes are cleaned annually and documented in the *Instrument Maintenance and Temperature Log* (SER-FORM-30) Binder.

REFRIGERATOR

a) Handling-See section 9.6.8.1.
b) Transport- section 9.6.8.1.
c) Storage- section 9.6.8.1.
d) Use-freezer portion may be used to store Takayama solution for the advantage of a longer expiration date period.
e) Planned Maintenance- section 9.6.8.1.

9.12 HEMATRACE® AS A CONFIRMATORY TEST FOR BLOOD

9.12.1 SCOPE

The ABAcard® HemaTrace® test qualitatively detects hemoglobin and is a confirmatory test for forensic identification of blood[75].

9.12.2 REAGENTS, CHEMICALS, STANDARDS, & CONTROLS

REAGENTS

- Not Applicable

CHEMICALS

- ABAcard®-provided extraction buffer

STANDARDS

- Reference Material - Known Blood Standard (only for initial QC of a new Lot # of cards) See section 9.10.2 for additional information.

CONTROLS

- **Positive Control**: a positive control is run when new lot numbers are obtained as an initial QA/QC step. Results are recorded in the Reagent Logbook on HemaTrace® Card Quality Assurance Worksheet and in the case notes.

  Note: Though not required, the positive control may be prepared from a dilute blood sample so it can be verified that the test is valid at lower levels of hemoglobin.

  1) The positive control could be made by adding 50µl whole blood to 1950µl of the supplied buffer.
  2) This solution could then be added to filter paper and dried.
  3) Small samples, approximately 1mm^2 in size, could be used as positive controls.

- **Negative Control**: a negative (reagent) control is performed alongside a positive control when new lot numbers are obtained as an initial QA/QC step and is required daily, as necessary[76]. Results are recorded in the Reagent Logbook on HemaTrace® Card Quality Assurance Worksheet and in the case notes. A positive reaction of a negative control renders the test inconclusive. See section 9.6.2 for additional information.

[75] Specificity: Human, Higher Primate, and Ferret blood
[76] Whenever HemaTrace® is going to be used to test stains on evidence, one negative control shall be run per lot# of cards used.

9.12.3 SAMPLE PREPARATION

9.12.3.1 METHOD

HemaTrace® test cards should be stored in a refrigerator until day of use.

1) A sample (thread, scraping, cutting, or etc.) of the suspected stain is treated by placing a small portion of the sample in the entire volume of the buffer in the supplied buffer tube. One tube is provided per test card.
2) Let the sample incubate at room temperature for at least 5 minutes (For samples that are older than 10 years, extend the incubation time to 30 minutes).
3) Label HemaTrace® test card with the appropriate sample ID and case number.
4) Briefly mix the sample. Add approximately 4 drops of sample with the supplied dropper to the sample well of the test card.
   Note: Should the sample inadvertently be deposited in the sample well along with the extract and buffer, the sample will not interfere with the test results.
5) Read results at 10 minutes. Results are **not** valid past 10 minutes.

9.12.4 QUALITY ASSURANCE/CONTROL MEASURES

As with all Serology Unit methodology, using clean techniques when conducting the HemaTrace® test on possible bloodstains is imperative to the procedure. The Serology Training Program covers all aspects of the proper techniques to test possible bloodstains.

Daily negative QC checks are performed on any Lot #s of HemaTrace® cards and supplied extraction buffer that are to be used on casework. This ensures that the cards are working properly as well as demonstrates the absence of contamination in the buffer. A positive QC check is performed on new Lot #s of HemaTrace® cards to demonstrate their reliability on producing the expected result.

Due to the sensitivity of the HemaTrace® cards, a positive presumptive phenolphthalein test is required to be conducted first. This prevents misinterpretation of positive results[77].

[77] Sometimes skin may have occult blood that cannot be visualized, however due to the sensitivity of HemaTrace®, it might produce a positive result.

9.12.5 INTERPRETATION OF RESULTS

9.12.5.1 PRECAUTIONS

The HemaTrace® cards are manufactured with areas of pink dye-labeled antibodies. These areas of the card may appear shadowed when supernatant wicks across that zone. Analysts should only interpret the test area as being positive if actual pink dye is observed (regardless of intensity.) Brighter lit areas of the laboratory may aid in correct interpretation of these areas of the card.

9.12.5.2 POSSIBLE SOURCES OF ERROR

- Inadequate sampling of stain. This may lead to an over-dilution of a possible bloodstain, thus preventing a visible positive reaction.
- Insufficient soaking time. This may lead to a lack of sufficient extraction, thus preventing the required antigen-antibody reactions.
- Reading results beyond 10 minutes. HemaTrace® cards may leave an artifact in the test zone that is not a true positive due to the manufacturing process. Results should only be read at 10 minutes.

9.12.5.3 LITERATURE REFERENCES

- NFSTC – Testing of Body Fluids (Blood)
- ABAcard® HemaTrace® Product Insert
- *Forensic Biology*, 2nd Ed. Chapter 2, 12, & 13

9.12.5.4 CRITERIA FOR RESULTS

1) **Positive Results**: Two red lines, one in the test area and one in the control area indicates the test result is positive.
   Note: Due to the sensitivity of HemaTrace® Test Cards, a POSITIVE Phenolphthalein Presumptive Test is a prerequisite for HemaTrace® testing.
2) **Negative Results**: Only one red line in the control area indicates the test result is negative. A negative result may indicate:
   a) The test result is negative.
   b) Sample was not properly diluted. This can cause a 'High Dose Hook Effect', which may give false negative results due to the presence of high concentration of hemoglobin in a sample. If High Dose Hook Effect is suspected, retest using a 1:10 and 1:100 fold dilutions. However, if proper sampling procedures are followed, the high dose hook effect is not encountered in the Serology Unit of the ASCL and therefore, does not lead to false negative results.
3) **Invalid**: No red line visible in the control area indicates the test is invalid.

9.12.5.5 TROUBLESHOOTING

1) **Positive Control** produces a negative result during quality assurance testing. Notify Physical Evidence Section Chief of nonconformity in testing. Document in notes.
   a) Obtain a new positive control and retest using a new test cassette.
      - If testing of new positive control yields positive results, discard previous (old) positive control and record findings.
      - If testing of new positive control yields negative results, open a new box of HemaTrace® cards and test using a new cassette.
      - If testing of new positive control yields positive results, discard previous (old) positive control and record findings.
      - If testing of positive control yields negative results, notify Physical Evidence Section Chief of nonconformity of testing.
   b) Start a Quality Assurance Concern workflow as necessary.
2) **Negative Control** produces a positive result. Notify Physical Evidence Section Chief of nonconformity in testing. Document in notes.
   a) Select approximately five new test cassettes from the current lot and test with supplied buffer only.
      - If testing yields negative results then record findings.
      - If testing yields positive or inconclusive results, notify Physical Evidence Section Chief of the nonconformity in testing.
   b) Start a Quality Assurance Concern workflow as necessary.
3) **Invalid result**: Control line is not detectable. Notify Physical Evidence Section Chief of nonconformity in testing. Repeat assay with a new test cassette. Document in notes.

9.12.6 NOTES/DOCUMENTATION REQUIREMENTS

1) If the HemaTrace® test is positive, record the positive results in the case notes. Describe the stain in the case notes. A thorough description will include the location of the stain or a drawing showing the location of the stain and a measurement of the stain.
2) If the HemaTrace® test is negative, record the negative result in the case notes.
3) The results of the positive and negative controls are recorded in the case notes.
4) The HemaTrace® cassette test Lot# is recorded in the case notes.

9.12.6.1 ASSESSMENT OF RESULTS

- If the HemaTrace® test is positive, then the analyst can report that blood was identified on that item. The sample is then retained using a collection method as outlined in section 9.3 Collection of Stains for Further Testing.
- If the HemaTrace® test is negative, then the analyst can report that no blood was chemically identified on that item.

9.12.7 REPORT WRITING

See section 9.2.7 for general report writing information regarding Serology Autotext.

RESULTS CHART

The evidence item(s) from which stains were tested for blood and found to be positive will be listed in the Serology Chart under the "Results" section of the report.

REPORT FINDINGS

Using JusticeTrax®, for each positive (+) blood evidence item that was examined, the analyst will select "Identified" in the blood findings drop-down menu. This will populate the blood column on the report to read "Identified."

Using JusticeTrax®, for each negative (-) blood evidence item that was examined, the analyst will select "Negative" in the blood findings drop-down menu. This will populate the blood column on the report to read "Negative."

- In instances where non-human blood is suspected on a blood evidence item both Takayama and HemaTrace® tests may be performed. If the Takayama test is positive but the HemaTrace® test is negative the analyst will select "See Below*" in the blood findings drop-down menu. In the "Further Explanation of Results" section of the report, "*Blood was identified on Q#; however, a confirmatory test specific to higher primates, humans, and ferrets was negative" can be written.

RETAINED SAMPLES

Under the Retained Samples portion of the report, the analyst will use the appropriate retained samples listing(s) below:

- **Retained Items**: The appropriate Q#(s) will be listed on the "Item(s)…" line.
- **Retained Cuttings**: The appropriate Q#(s) will be listed on the "Cutting(s) from…" line.
- **Retained Swabs**: The appropriate Q#(s) will be listed on the "Swab(s) from…" line.

9.12.8 CRITICAL REAGENTS & EQUIPMENT SPECIFICATIONS

There are no critical reagents used in the HemaTrace® Test as a confirmatory test for blood.

9.12.8.1 CRITICAL EQUIPMENT

- ABAcard® HemaTrace® Test
  a) Handling- Gloves shall be worn when handling ABAcard® HemaTrace® test cards.
  b) Transport-If transporting cards, best practice is to leave the pouches sealed until reaching the testing location.
  c) Storage- ABAcard® HemaTrace® test cards shall be stored in the refrigerator overnight.

d) Use- ABAcard® HemaTrace® test cards are used as a confirmatory test for the presence of blood.
e) Planned Maintenance-none, however expiration dates shall be observed.

9.12.8.2 EQUIPMENT

REFRIGERATOR

a) Handling-See section 9.6.8.1.
b) Transport- See section 9.6.8.1.
c) Storage- See section 9.6.8.1.
d) Use-for climate control storage of ABAcard® HemaTrace® cards and buffer.
e) Planned Maintenance- See section 9.6.8.1.

9.13 SEXUAL ASSAULT KIT PROCESSING GUIDELINES

Sexual Assault Kits may be submitted as routine, ACT 1168, or SAFER kits, (depending on the age of the kit at the time of their submission to the laboratory), however, processing of these kits will be the same.

These guidelines bypass body fluid identification of kit contents in order to expedite the submission of kit samples to the Forensic DNA Section where they are analyzed for possible foreign male DNA (depending on case scenario).

Sexual assault cases will routinely be analyzed in the following order: (1) Sexual assault kit, (2) Underwear, (3) Clothing, (4) Bedding. Regardless of kit contents, other submitted items will generally not be examined initially by Serology. These items may be examined at a later date based upon communication/request by the DNA section or the customer.

Exceptions to this include expedited cases submitted with a sexual assault kit and other evidence items. In such cases, the Physical Evidence and DNA Section Chiefs will determine which items to initially work. Refer to the *ASCL Case Management Guidelines* (ASCL-DOC-10) document for more information.

9.13.1 SEXUAL ASSAULT KITS–ROUTINE PROCESSING

State of Arkansas Sexual Assault Kits (SASAEC kits) are supplied with the following items for collection by medical staff:

- Vaginal Swabs (+ envelope)
- Oral Swabs (+ envelope)
- Rectal Swabs (+ envelope)
- Underwear bag
- Pubic Hair Combings collection supplies (comb, exam paper, + envelope)
- Known Blood Sample card (+ envelope)

Note: The Pubic Hair Combings will **not** routinely be examined but will remain in the kit. It will be documented as "also submitted, not examined (ASNE)" in the analyst's notes.

KIT SAMPLES

Swabs: Kit swabs will not routinely be examined for the presence of semen or blood, but rather will have portions cut into appropriate DNA extraction tubes and will be forwarded to the Forensic DNA section for extraction. The correlating swabs will also be retained and submitted as well.

Underwear: The Underwear will not be routinely tested for the presence of semen or blood. They will generally be tape lifted and visually examined with an ALS. Tape lifts will be retained in large manila envelope. If a sanitary pad/napkin is received with the underwear they will be treated as one item. If no underwear are received and only a sanitary pad/napkin is received it will be worked in the same way as the underwear.

All fluorescing stains and red-brown/brown stains will be marked and retained. Only the single best stain (typically the gusset/crotch area) will have a portion cut into an appropriate extraction tube and the stain cutting also retained as "Q#-1S". All other stains will be retained as Q#-2S, 3S, etc. If a female suspect is described, the best stain will be retained as Q#-1T and all other stains as Q#-2T, 3T, etc. (document in notes.)

If no fluorescence of visual staining is observed, a center portion of the gusset/crotch will be marked and retained as "Q#-1S" (Q01-1T if female suspect if described) in a coin envelope. A portion will be cut into an appropriate extraction tube.

Known Blood Sample: Retain portion in the appropriate DNA extraction tube and retain the remaining sample in a coin envelope.

Miscellaneous Items: Periodically, hospital examination paper/pads are received in the sexual assault kits. These items will **not** routinely be examined, but will remain in the kit. They will be documented as "also submitted, not examined (ASNE)" in the analyst's notes.

In addition, any tampons and sanitary pads/napkins (received separately from the underwear) submitted in the sexual assault kit will be retained in a 3.5" x 6.5" manila envelope inside of a large manila envelope. They will not be tested for the presence of semen or blood. Exceptions to this is when a sanitary pad/napkin is received in a kit that does not contain underwear (see "Underwear" section above for more information).

SAMPLE NAMES

Name the swabs as they are labeled by the medical staff. If a differentiation is made between swabs in the same envelope, they will be handled/retained separately (i.e., inner vaginal swabs-cut into tube; outer vaginal swabs-retained only).

Note: Out-of-State sexual assault kits from neighboring States may be submitted with additional contents. Use the chart below to determine which swabs and samples should be processed.

The following chart serves as a general guideline for the handling of different samples that may be included in a sexual assault kit. If an analyst receives a sample that isn't listed in the chart, consultation with either the Physical Evidence Section Chief or the Forensic DNA Section Chief(s) may provide direction:

Sample Name	Flip-Top Tube (differential extraction)	Screw-Top/Skirted Tube (modified extraction)
Vaginal(inner) or Cervical Swabs	**X** (male suspect)	**X** (female suspect)
Penile Swabs		**X**
Rectal Swabs	**X** (male suspect)	**X** (female suspect)
Known Samples		**X**
Oral Swabs	Retain Swabs ONLY in coin envelope <u>Exceptions:</u> • **X** (male suspect) - Cut if investigating agency specifically requests analysis prior to kit examination. • **X** (male suspect) - Cut if victim ONLY alleges an oral assault. Vaginal/Penile and Rectal swabs (if collected) will also be cut in these instances.	
<u>ADDITIONAL**</u> <u>Genital Area Swabs</u> (e.g., Vaginal (outer/external), labial, vulvar, mons pubis, posterior fourchette, etc.) **These are only considered *additional* if Vaginal (inner) or Cervical Swabs weren't submitted. (If so, cut at least one of these sites into a flip-top tube.)	Retain Swabs ONLY in coin envelope	
Miscellaneous/Extra Body Swabs	Retain Swabs ONLY in coin envelope.	
Speculum Swabs	Retain Swabs ONLY in coin envelope	
Fingernail Scrapings/Clippings	Retain ONLY in original env.	

Tampons (Refer to section 9.14.2)	Retain ONLY in 3.5“ x 6.5“ manila env.	

DNA EXTRACTION TUBES

Flip-Top Tubes-used for Differential Extractions.

For possible semen samples (see chart above)

Screw-Top Tubes (or Skirted Tubes)-used for Modified Extractions.

For Penile Swabs & known/reference samples

CUTTING SIZE GUIDELINES

1) Questioned Swabs
   a) For DNA analysis
      - Label each tube with case # (i.e., 2019-XXXXXX) and item number (Q#) on extraction tube sticker labels
      - If there are 4 swabs, cut ~1/4 of each swab (top to bottom of swab tip) from each swab (to roughly equal one total swab quantity) into a tube.
      - If there are more than 4 swabs, adjust cutting size smaller per swab.
      - If there are 2 swabs, cut ~ ½ of each into a tube.
      - If only 1 swab is collected, cut ~ 3/4 of the swab into a tube.
   b) For general retention
      - Cut or snap off the swab sticks and place the remaining portion of each swab set in a coin envelope and label with case number, item number (Q#), and initials. Tape seal the envelope and initial seal.
2) Known Samples
   a) For DNA analysis
      - Label each screw-top tube with the case # (i.e., 2019-XXXXXX) and item number (K#) on an extraction tube sticker label.
      - The provided card has four circled areas. If blood saturates all areas of the circles, a generous 1/8 of one circle’s stain should be cut and placed into the appropriate extraction tube.
      - If only small dots of blood are present, cut most (but not all) out and place them in the extraction tube.
   b) For general retention
      - The remaining card (with remaining stain) will also be retained in a labeled coin envelope. Tape seal and initial seal.
   c) For known oral swab(s) received outside of the kit (i.e., suspect known oral swabs)
      - Label a screw–top tube with the case number (i.e., 2019-XXXXXX) and item number (K#) on an extraction tube sticker label.
      - Cut ~1/8 of a swab into the extraction tube.

- Cut or snap off the swab stick(s) and place the remaining portion of the swab(s) in a coin envelope and label with case number, item number (K#), and initials. Tape seal and initial seal.

3) Underwear

Underwear will not be chemically tested for semen or blood.

a) For DNA analysis

- The best/most probative stain will be retained and cut into an extraction tube. Retain cutting(s) that equal ~ ½" square (i.e., 4 cuttings with each ~1/8th" square in size from areas within the length of the stain) and place in flip-top extraction tube. A cutting that is at least ½" square will also be retained in a labeled coin envelope. Tape seal and initial seal.
- Female suspect cases- retain cuttings from the inner fly area (for male victims) or from the inner crotch/gusset area (for female victims) according to the size guidelines above. Place in a screw-top extraction tube. A cutting that is at least ½" square will also be retained in a labeled coin envelope. Tape seal and initial seal.

b) For retention of other stains

- From the remaining stain(s) on the garment, retain a cutting that is at least ½" square in a labeled coin envelope. Tape seal and initial seal.

4) Storage information

- ONLY cut into a tube the samples that are to initially be processed by the DNA section. The DNA analysts must extract what we place in tubes or the samples may mold. Additional samples can be cut into tubes by the DNA section, if warranted.
- Extraction tubes will remain in the analyst's tube rack/holder until they are transferred to FD in PE Secure Storage.
- Coin envelopes containing the parent cuttings and other retained samples will be stored in 3.5" x 6.5" manila envelope(s).
- The chain of custody of the extraction tubes will be maintained via the parent cuttings.
- The manila envelope(s) will be electronically transferred to FD in PE Secure Storage. The envelope(s) and the corresponding extraction tubes (in the analyst's tube rack/holder) will then be physically transferred to FD in PE Secure Storage. The envelope(s) will be filed and the extraction tube will then be transferred to the tube box/holder located in FD in PE Secure Storage.

***Note*:** During the evidence transfer process, handle extraction tubes with a clean glove to prevent contamination.

ADDITIONAL ORAL SAMPLES SUBMITTED IN KIT

Any additional oral samples submitted to serve as a victim DNA known (*e.g.*, buccal swabs, oral sample, saliva swabs) can be given a K#, cut into a screw top tube, and related to the DNA request, ***if*** the following applies: **there is clear hospital/center documentation that significant time has**

passed after collecting the questioned oral swabs (in the provided oral swab env.) and/or oral rinsing or brushing has occurred at the hospital/center.

Note: Patient-reported hygiene activities do not qualify the sample.

Any additional oral samples submitted to serve as a victim DNA known (*e.g.*, buccal swabs, oral sample, saliva swabs) **with no supporting information** as described above, will be retained as a questioned item and assigned a Q#. These samples should NOT be cut into an extraction tube nor related to the DNA request.

LIMS

When itemizing samples in JusticeTrax®, it is only necessary to itemize one level of each sample (e.g., Vaginal Swabs). Do not itemize a separate extraction tube level, as they are considered work product. The extraction tube and swabs are both stored in FD in PE Secure Storage.

The Sexual Assault Kits will be worked under a "Serology SA Processing" request. Once all samples have been retained/stored and notes have been completed and scanned into the Serology SA Processing request folder in the Attachments tab, mark the request as draft complete. There will be no report created and no review process for this request type.

Create a "DNA SA Processing" request and relate the sample(s) being submitted for DNA analysis to this request. The sexual assault kit and any unexamined bulk evidence should also be related to this request.

MEDICAL RECORDS & CONSENSUAL PARTNERS INFORMATION

The medical records received in the sexual assault kit will be scanned into the subfolder labeled with the victim's name within the "Individuals" folder in the Attachments tab. The Physical Evidence Section will not contact the investigating agency to request known samples from consensual partners or to clarify slight spelling discrepancies between the victim's name on the Sexual Assault Kit/medical records and the Arkansas State Crime Laboratory Evidence Submission Form.

9.13.2 ACT 1168/SAFER SEXUAL ASSAULT KITS

ACT1168/SAFER sexual assault kits are *generally* over one year old on the date submitted to the ASCL. These types of cases are *generally* not active and are not going to be prosecuted, but due to AR State Law, will be tested by the laboratory. Processing of these kits will follow the same procedures as listed in section 9.13.1 Sexual Assault Kits-routine processing.

9.14 MISCELLANEOUS

9.14.1 SEPARATELY PACKAGED CONTROL SWABS

Control swabs are sometimes submitted in cases. Mostly, these are cases where blood testing is warranted. If an agency submits a questioned swab from an item in one envelope and a control swab in another envelope, retain the control swab if you are retaining the questioned swab. It is not a requirement to test the control swab presumptively for blood, but the analyst may choose to do so. The analyst may also choose to conduct a visual examination only and write nothing of value noted (NOVN) for blood in their notes. For either option, report test results accordingly for the control swab[78].

9.14.2 TAMPONS

Tampons submitted as evidence in a Sexual Assault Kit will not routinely be tested for bodily fluids. The analyst will assign an appropriate Q# and retain the item in a 3.5" x 6.5" manila envelope, stored in a large, tape-sealed manila envelope, and labeled appropriately. Document in notes.

If a tampon is the *only* item submitted in a case, semen testing will be performed. If a tampon is submitted along with other case items and there is specific case information that warrants testing, the tampon may be examined for the presence of semen. Medical paperwork may be helpful in determining locations of optimal value for sampling based on if the item was placed before or after incident. If semen testing is warranted, refer to section 9.7 test methods, as acid phosphatase testing is typically not useful with tampons.

Reporting:

Relate the tampon to the Results chart on the report. If no serological testing was conducted, use the "Not Tested" option for blood and/or semen on the report.

9.14.3 CONDOMS

Condoms submitted as evidence will not routinely be tested for semen, but will be handled similarly to kit swabs. (See section 9.13.1.) Blood testing is not mandatory, but may be conducted on condoms not received inside a sexual assault kit if the case information warrants it (i.e., prepubertal child, male victim, postmenopausal or elderly, documented medical trauma resulting in bleeding).

Procedural steps:

[78] Either "Negative" or "no visual stains" for Blood

- Swab the outer surface of the condom (as received) and allow swab(s) to dry. Document retention in notes. Store in a labeled, tape-sealed and initialed coin envelope as "Q#-1S outer, as received."
- Swab the inner surface of the condom (as received) and allow swab(s) to dry. Document retention in notes. Store in a labeled, tape-sealed and initialed coin envelope as "Q#-2S inner, as received."
- Cut a large portion from the tip of the condom and store in a labeled, tape-sealed and initialed coin envelope (e.g., Q#-TIP). Document retention in notes.

Reporting:

Relate the condom to the Results chart on the report. Use Edit Findings to report any blood testing that may have occurred. If no serological testing was conducted, use the "Not Tested'' option for blood and/or semen on the report.

Urine/Fluids

Occasionally, urine or other liquid samples are submitted as evidence items intended to be tested for the presence of semen/male DNA. Sometimes urine samples are submitted for the intention of Toxicological analysis in addition to Serological analysis. In these situations, care will be taken to preserve the specimen. Urine/fluid samples will not routinely be tested for blood.

Procedural steps:

- Filter entire liquid specimen using filter paper, clean funnel, and clean beaker. (If total volume is significant, it may be necessary to set up more than one filtering apparatus.)
- Cut a portion of the center of the filter paper into a centrifuge tube. Refer to section 9.7 for testing procedures.
- If semen result is negative, properly dispose of filter paper in biohazardous waste.
- If semen result is positive, allow the filter paper to dry, and then place in a labeled coin envelope.

Note: The liquid specimen is considered the "parent item" in the LIMS, and will be assigned a Q#. If the filter paper is retained, it should be itemized from the liquid specimen as a Q#-1S sample.

Reporting:

Relate the liquid specimen to the Results chart on the report. Use Edit Findings to report semen testing results, according to section 9.9.7. For the blood column, use the "Not Tested" option for the report

9.14.4 MEDICAL EXAMINER SUBMISSIONS TO PHYSICAL EVIDENCE

Generally, the Medical Examiner Section will submit any sexual assault swabs, neck swabs, swabs from hands/fingernails, and fingernail scrapings/clippings directly to the Forensic DNA section. If there is a specific need for body fluid testing, it should be marked as such by the submitter.

Clothing items submitted to Physical Evidence (PE) should be reviewed first to determine if distance determination (GSR) is needed. If not, proceed with appropriate section 9.4 examinations and appropriate presumptive testing. Consultation with the investigating officer may be helpful in determining whether any blood testing should be conducted or if there are specific areas of interest for touch DNA.

Ligatures shall be examined following applicable section 9.2 and section 9.3 protocol for collection of hairs, fibers, and possible touch DNA/skin cells. Photographing any knots, as received, is encouraged for documentation.

Bags from Hands are occasionally submitted to PE. Document in notes and visually examine for hairs. If found, retain the hairs on tape lifts and store in PE Secure Storage. If no hairs are observed, document their absence in notes, and return bags with bulk evidence.

9.14.5 DNA DATABASE KITS

Occasionally, DNA Database Kits are submitted with bulk evidence to Serology. Contact CODIS to determine whether it is a sample that they need.

- **If needed by CODIS**: Itemize/Decontainerize the kit from the bulk evidence on JusticeTrax® and barcode the kit. Transfer to FD Secure Storage and place in the appropriate box at the bottom of the cabinet. Document in notes.
- **If NOT needed by CODIS**: Retain the paper portion of it in a labeled (e.g., K# - Known Oral Sample from [name]), tape-sealed coin envelope and store in a DNA envelope along with any other retained questioned items. Document in notes.

9.14.6 PROPERTY CASE EVIDENCE

Serological testing will not be routinely performed on Property Crime evidence[79]. Instead, touch DNA and/or possible blood samples will be collected and forwarded to the DNA Section for processing. Notes and appropriate documentation will be maintained in the LIMS, but a report will not typically be generated.

Procedural Steps:

[79] On occasion, a property case may need Serological testing and a report issued. This will be determined by the Physical Evidence Section Chief. In this event, samples will be examined according to appropriate test methods in section 9 of this manual.

- Identify most probative items and those that meet *Case Management Guidelines* (ASCL-DOC-10).
- Select one or two of the most probative items for testing.
- Document packaging descriptions, item descriptions, and stain descriptions if applicable. Refer to section 9.1.1 for general guidelines.
- Collect any possible blood stains and/or possible touch/transfer DNA samples. Do not collect tape lifts.
- Retain or return samples and document appropriately:
  - Retain only samples being sent to DNA (one or two questioned samples per case) plus any known samples.
  - Retain all control cuttings.
  - Return samples (swabs, cuttings, etc.) that are not being sent to DNA.
    - Return these to the agency with the appropriate evidence items/packaging.
- Itemize appropriately in the LIMS. Preserve notes/documentation in the LIMS under the appropriate Serology request.
- Create a DNA request and relate appropriate samples. Cancel the Serology request.